Induction of meiotic gynogenesis in the stinging catfish Heteropneustes fossilis (Bloch) and evidence for female homogamety

Article *in* Aquaculture Research · December 2010

DOI: 10.1111/j.1365-2109.2010.02593.x

CITATIONS
20

READS
162

3 authors, including:

Godwin Christopher
VIT University
44 PUBLICATIONS **392** CITATIONS

SEE PROFILE

Navaneeth Sukumaran
Lucas - TVS
11 PUBLICATIONS **146** CITATIONS

SEE PROFILE

Aquaculture Research

Aquaculture Research, 2010, **42**, 129–138 doi: 10.1111/j.1365-2109.2010.02593.x

Induction of meiotic gynogenesis in the stinging catfish *Heteropneustes fossilis* (Bloch) and evidence for female homogamety

John Samuel Godwin Christopher[1], Arunachalam Ganesan Murugesan[2] & Natarajapillai Sukumaran[3]

[1]School of Bio Science and Technology, Vellore Institute of Technology University, Vellore, India

[2]SPK Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, India

[3]School of Life Sciences, Vels University, Pallavaram, Chennai, India

Correspondence: J Godwin Christopher, School of Bio Science and Technology, Vellore Institute of Technology University, Vellore 632 014, India. E-mail: godwinj@vit.ac.in

Abstract

This study reports the results on induced meiotic diploid gynogenesis and female homogametic nature in the Indian catfish, *Heteropneustes fossilis*. The eggs of *H. fossilis* were inseminated with conspecific sperm. The sperm suspension was diluted to 1×10^7 sperm mL^{-1} in Hanks balanced salt solution. Sperm were irradiated under UV light, with the exposure time ranging from 15 to 360 s (7500 ergs mm^{-2} for 60 s). The genetic inactivation of paternal chromosomes was confirmed by chromosome counting from the larval cells and the larvae also had a characteristic haploid syndrome. A typical 'Hertwig effect' in the yield of hatched larvae was observed with doses of UV exposure >75 s (9375 ergs mm^2). Larvae resulting from sperm UV irradiated above 120 s (15 000 ergs mm^2) were 100% haploids. Application of heat shock to the activated eggs was effective in suppressing the release of the second polar body (meiotic gynogenesis) and resulted in diploid gynogenetic larvae morphologically identical to those of the control. The best yield of diploid gynogens (49.3% with respect to the control) was found to be at 6 min after egg activation and the heat shock at 41 °C for a 1-min duration, at an ambient water temperature of 27 °C. A total of 113 diploid gynogenetic fry from seven different female fish were reared and subjected to sexing. All gynogenetic fish were female in contrast to the control, which had a mean sex ratio of 56.7% females (which was not significantly different from 50% female). From these results, the sex determination mechanism in *H. fossilis* was presumed to be female homogamety.

Keywords: *Heteropneustes fossilis*, UV irradiation, heat shock, meiotic gynogenesis, sex ratio, sex determination

Introduction

Heteropneustes fossilis (stinging catfish) is a commercially important freshwater catfish distributed in the tropical waters of the Indian sub-continent. *Heteropneustes fossilis* is highly prized for its low fat, high protein and high iron content. It adapts well to water bodies with low dissolved oxygen and higher stocking densities (Dehadrai, Yusuf & Das 1985), and its air breathing apparatus enables it to be sold as 'live fish' in the market. In *H. fossilis*, there are considerable studies on the reproductive biology (Saxena & Sandhu 1993), breeding and cryopreservation of sperm (Lal, Barman, Punia, Khare, Mohindra, Lal, Gopalakrishnan, Sah & Lakra 2009), but only very few studies on genetic manipulation (Tiwary, Kirubagaran & Ray 1997, 2000; Gheyas, Mollah & Hussain 2001) are available.

Gynogenesis is a form of uniparental chromosome inheritance from female. The techniques are relatively simple. Generally, UV-irradiation is provided to genetically inactivate the spermatozoan DNA, while keeping the spermatozoa alive and motile. The treated spermatozoa can then be used to activate developments in egg without the transfer of paternal genes. The optimal UV exposure duration needs to be determined for the successful production of haploid embryos. Haploid larvae exhibit the 'haploid syndrome' (Purdom 1969; Varadaraj 1993; Christo-

pher, Murugesan & Sukumaran 2009) and usually die after hatching due to the expression of harmful recessive genes (Thorgaard 1983). Diploid condition by duplication of the haploid set of egg chromosomes can be achieved by shock treatment. The production of meiotic gynogens is easier than mitotic gynogens, but it is very difficult to establish complete homozygous lines by meiotic gynogenesis due to gene–centromere recombinations (Thorgaard, Allendorf & Knudsen 1983); however, it may be possible to produce isogenic lines by two, three or more cycles of meiotic gynogenesis, because the distal and proximal loci on homologous chromosomes would be fixed in the heterozygous and homozygous conditions respectively. Thus, meiotic gynogenesis is a more practical method to fix desirable genotypes for the rapid improvement of aquaculture strains as in rainbow trout (Thorgaard *et al.* 1983).

Gynogens has vast potential applications in genetic research and aquaculture. Gynogenesis, an induced developmental process with the maternal genome alone, is a vital tool for the establishment of inbred isogenic lines of fish for fish genetics research and breeding, inheritance of desirable maternal characteristics, identification of the sex-determination system and generation of all-female progeny (You, Yu, Tan & Tong 2008).

Gynogenesis in combination with sex reversal produces neomales for monosexing, and triploidization of gynogenetic females produces an XXX female. Gynogenesis and triploidy to produce all-female sterile populations (Quillet, Foisil, Chevassus, Chourrout & Liu 1991) are commonly practiced in private trout farms throughout Japan (Arai 2001). All-female and/or triploid salmon are widely cultured in Canada (Benfey 1996) and on a smaller scale also in Japan (Arai 2001).

Gynogenesis has been induced successfully in a large number of finfish species such as African catfish, *Clarias gariepinus* (Volckaert, Galbusera, Hellemant, Van den Heute, Vanstaen & Ollevier 1994; Varadi, Benko & Varga 1999), *Clarias macrocephalus* (Na-Nakorn, Rangsin & Asunkul 1993), channel catfish, *Ictalurus punctatus* (Goudie, Simco, Davis & Liu 1995), silver barb, *Puntius gonionotus* (Pongthana, Penman & Karnasuta 1995), and in many other species. Gynogens have been periodically reviewed by many authors, Purdom and Lincoln (1973), Purdom (1983), Thorgaard and Allen (1987), Ihssen, Mckay, McMillan and Philips (1990), Pandian and Koteeswaran (1998), Hulata (2001), Komen and Thorgaard (2007), etc. In fish, it is rarely possible to characterize sex determination cytogenetically due to the low occurrence of heteromorphic sex chromosomes (Devlin & Nagahama 2002). Gynogenesis can also be used for the elucidation of genetic sex determination modes in only one generation (Stanley 1976; Purdom 1983). All the gynogenetic offspring would be female in a species with female homogamety (XX) and a 1:1 male to female ratio if the female fish show heterogamety (ZW). The sex-determination system has been well studied in common carp, *Cyprinus carpio* (Nagy, Bercsenyi & Csanyi 1981), rainbow trout, *Oncorhynchus mykiss* (Bye & Lincoln 1986), silver carp, *Hypophthalichthys molitrix* (Mirza & Shelton 1988), rosy bitteling, *Rhodeus ocellatus ocellatus* (Kawamura 1998), Atlantic halibut *Hippoglossus hippoglossus* (Tvedt, Benfey, Martin-Robichaud, McGowan & Reith 2006), and Taiwaninmutakala *Paramisgurnus dabryanus* (You *et al.* 2008). No report has been made available on the sex-determination system of this Indian catfish *H. fossilis*.

The objective of the present study is to optimize conditions for the induction of meiotic diploid gynogenesis in *H. fossilis*, applying heat shock to eggs activated with UV – ray-irradiated sperm, besides elucidating the sex-determination mechanism by the sex ratio.

Materials and methods

Brood stock collection

The experimental fish, *H. fossilis* (male 40–80 g body weight (BW) and female fish 80–120 g BW), were collected during the month of June 2006 (early spawning phase) from the ponds in and around Alwarkurichi, Tamil Nadu (8°48′00″N; 77°27′30″E). On arrival to the laboratory, fish were disinfected with formalin (50 ppm) for 2 h to remove external parasites and pathogens. Matured female brood fish were primarily selected on the basis of a soft and bulging belly, which yielded continuous ova of uniform size on gentle stripping. Then these eggs were examined under a stereozoom microscope to checking their maturity. The selected fish were stocked in a polyvinylchloride tank ($3 \times 2 \times 1$ m) with continuous aeration, a normal photoperiod (13.5 h L:10.5 h D) and ambient water temperature (26 ± 2 °C) to induce final ovulation.

Induction of ovulation

The induction of final maturation and ovulation was stimulated using a single intramuscular injection of OVAPRIM at a rate of 0.3 and 0.5 mg kg^{-1} BW for males and females respectively (Christopher *et al.* 2009).

Collection of gametes

The eggs were collected 10 h after an ovaprim injection at an ambient water temperature of 26 ± 1 °C (determined by pilot studies). A gentle press on the abdomen towards the anus yielded a copious stream of transparent greenish brown eggs. These eggs were collected in a glass bowl previously rinsed with Hanks balanced salt solution (HBSS). The male fish were anaesthetized and sacrificed for the collection of milt. The testes were surgically removed and cut into small parts, and then gently squeezed in a glass tissue homogenizer with a few drops of HBSS. The left-out tissue particles were removed and the milt was diluted to 1:10 with HBSS. The sperm motility was checked for each sample by taking an aliquot of 10 μL in a microslide and activated by 3 μL of tap water. Samples with high motility (97%) alone were used for the experiment. The sperm suspension was prepared 1 h before stripping of eggs and kept in a refrigerator at 4 °C. The sperm of *H. fossilis* remain viable for more than 6 h at 4 °C (Rexlin 1996).

Genetic inactivation of sperm

A simple UV irradiation chamber (Plate 1) was designed with four UV C, 15 W germicidal lamps (Philips, Eindhoven, the Netherlands). Two lamps were fixed on the dorsal and two on the ventral sides at a distance of 27 cm from the centre, where the sperm suspension was placed for irradiation. The sperm suspension of *H. fossilis* was diluted in the HBSS to obtain a sperm count of $\sim 1 \times 10^7$ cells mL^{-1}. This diluted sperm was placed on a thin (25 μm) transparent film dish to a height of 0.5 mm and placed at the centre (between the dorsal and the ventral lamps) for UV irradiation. The entire process was carried out in the dark to avoid genetic photo-reactivation of sperm (Kaastrup & Horlyck 1987). To determine the extension point of sperm irradiation, the post irradiation motility rates were assessed at regular intervals of 15 s (Christopher 2001).

Sperm motility assessment

Sperm motility was assessed using a simple motility test. A part of the sperm suspension was activated with water and a visual assessment of motility was performed under a microscope, yielding a score for each sample viz. 0 (no sperm motility) 0.5, 1.0, 1.5, 2.0, 2.5 and 3 (all sperm moving vigorously). This was observed by two different observers. Sperm with different UV exposure times were used for egg activation to assess the complete genetic inactivation. A maximum UV irradiation dose with high motility alone was used for activating the eggs.

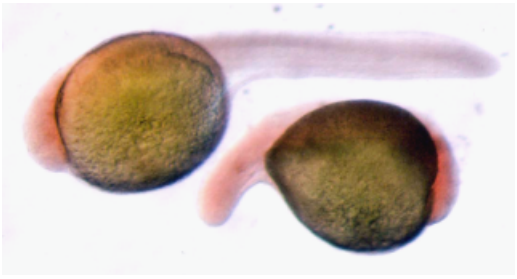

Plate 1 Comparison of *Heteropneustes fossilis* hatching. Normal appearance of meiotic diploid gynogen (2n), strait tail with that of haploid (n) bend, curved and short tail.

Haploid gynogenesis

Different groups of eggs were inseminated with non-irradiated (control) and irradiated sperms (15–360 s). These activated eggs (with different dosages of irradiated sperm samples) were incubated in individual rectangular trays of 25 × 20 × 5 cm (L × B × H) and the water in the trays was gently exchanged by siphoning, without disturbing the eggs, every 6 h until hatching. The survival percentages of inseminated eggs were scored for all hatchling. Each treatment group of eggs generally consisted of a total of 800–1200 eggs including duplicate. A sample of 40–50 eggs were siphoned out using a filler and placed in an embryo cup to assess the developmental stages under a steriozoom microscope (Nikon SMZ-U, Nikon Instech, Kanagawa, Japan). Two replicate experiments were carried out. The survival rates in these experiments were calculated relative to those of the controls (non-irradiated sperm-positive control) of the same experimental batch. Haploid individuals were confirmed by chromosome counting as per the methodology of Kligerman and Bloom (1977). At least 20 karyotypes were counted per individual and 10 individuals were sampled from each group.

Diploid gynogenesis

The irradiated sperm that yielded 100% haploids with a high motility score alone were used for inseminating eggs. Eggs activated with such UV-irradiated sperm were subjected to a wide range of heat shock at different temperatures of 38, 39, 40 and 41 °C for different post activation times from 1 to 8 min and

for different durations of 1 to 3 min to induce diploidy via retention of the second polar body (meiotic gynogenesis). For each heat shock treatment, 800–1200 eggs were used. All the eggs in each batch belonged to a single female and the percentage calculation was performed according to its positive control. The ambient water temperature throughout the experiment was 27 °C. The heat-shocked eggs were transferred directly to small rectangular trays and incubated until hatching. The water in the tray was exchanged gently by siphoning, without disturbing the embryo, every 6 h.

Hatchlings were observed under a stereozoom microscope for diploid confirmation. The hatchlings with a normal appearance similar to the control were the primary criterion to estimate the success of diploidization, followed by chromosome counting.

Rearing and sex ratio in diploid gynogenetic fry

Diploid gynogenetic fry derived from the seven different female fish were reared separately along with its control. A total of 193 diploid gynogenetic fries (without deformities) were selected. Initially, the fry were held in 5 L rectangular trays for 30 days. For the first 20 days, the fries were fed with boiled egg yolk and then with artemia. The water was exchanged every 12 h. After 30 days, they were transferred to 1 m fibreglass tanks and reared for the remaining 3 months. The young ones were fed with filtered *Moina* spp. for 3 months and then shifted gradually to formulated feed with 40% protein. Small pieces of broken pots were placed inside the trays and tanks throughout the rearing period for the fry to hide underneath.

Fish sexing was based on morphological characteristics, where males have projected anal papillae and females have an anal opening. The sex of fish was confirmed by dissecting the gonad and observing under a stereozoom microscope (Nikon SMZ-U).

All data were standardized to the relative percentage (i.e. percentage survivals of the embryos of the treated groups and haploid control groups were expressed relative to the diploid positive control from the same female) to reduce the maternal effect among the experiments, as described by Volckaert *et al.* (1994). Consequently, the fertilization rate of positive controls was always 100%, although the actual fertilization rate could not reach 100%. All the relative data were arcsin-transformed and tested for significance ($P < 0.05$) using one-way analysis of variance (ANOVA).

Comparisons of the observed sex ratios against the expected ratio (1:1) were performed using the χ^2-test (Zar 1984).

Results

UV irradiation and haploid gynogenesis

The data on the effect of UV-irradiation on sperm motility are presented in Fig. 1. It is clear that the motility declined as the duration of UV irradiation increased. However, there was no considerable change in motility up to 90 s of UV irradiation.

The survival of embryos, hatching and the percentage of haploids obtained by the sperm subjected to UV irradiation for different durations are shown in Fig. 2. The survival of embryos and hatching percentage decreased at 60 and 75 s of exposure (7500 and 9375 ergs mm $^{-2}$). However, both survival of embryo

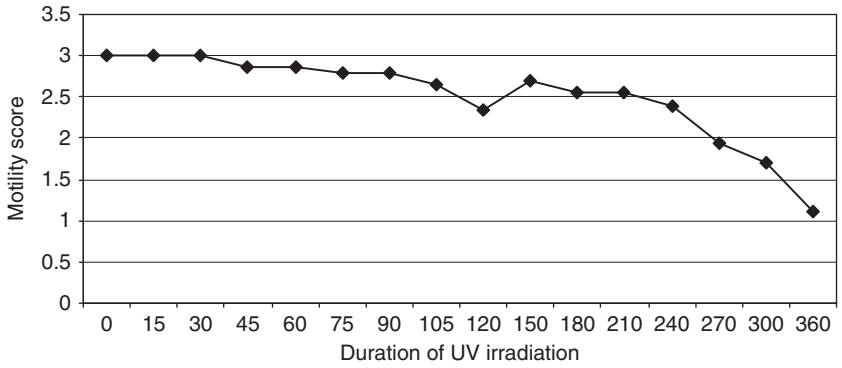

Figure 1 Effect of UV irradiation on sperm motility of *Heteropneustes fossilis*.

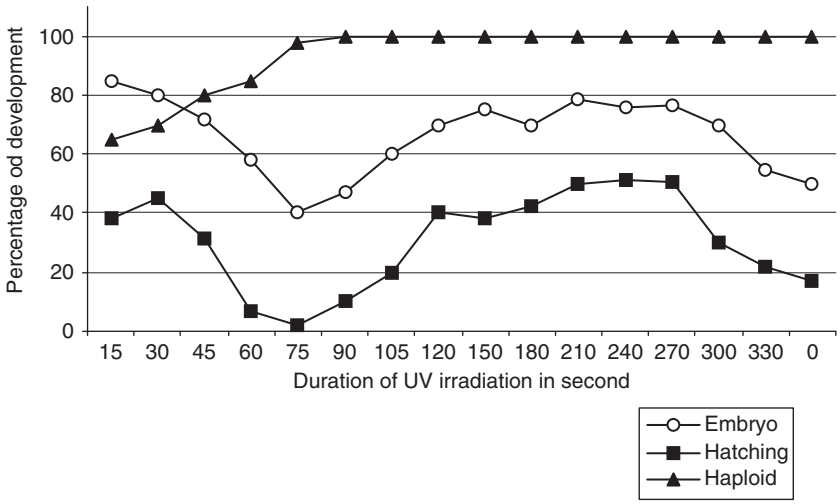

Figure 2 Changes in the survival of embryo, hatching and the percentage occurrence of haploids in the *Heteropneustes fossilis* eggs, inseminated with UV-irradiated sperm for different durations of time.

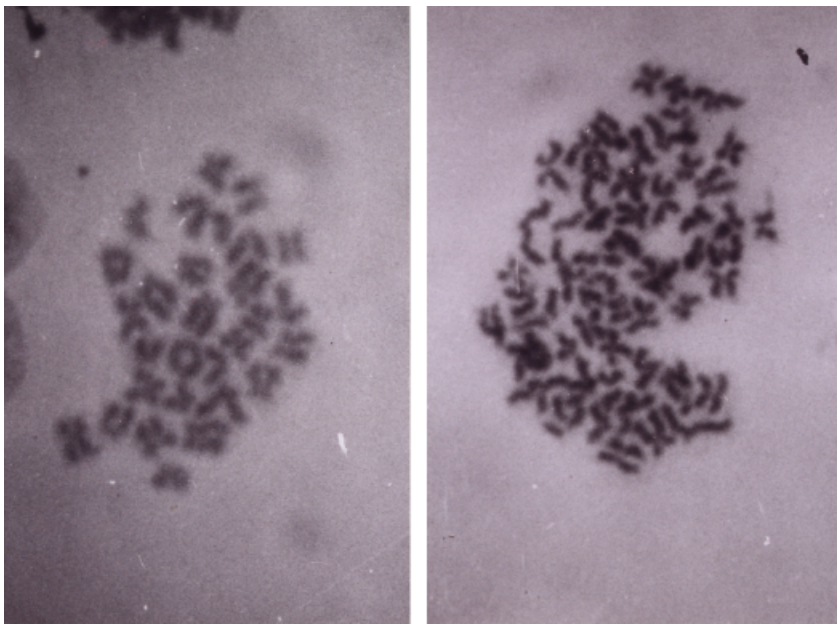

Plate 2 Chromosome spread of haploid with 29 chromosomes and diploid meiotic gynogen with 58 chromosomes of *Heteropneustes fossilis*.

and hatching percentage increased again from 90 to 270 s of UV irradiation, and once again the corresponding values showed a decreasing trend beyond 300 s of UV irradiation.

The majority of the eggs inseminated with UV-irradiated sperm for <90 s died during the embryonic development. Almost all the eggs developed up to the blastula stage and a few were able to hatch out. But some larvae displayed various deformities, resulting in mortality. The hatched fry from the eggs inseminated with UV-irradiated sperm for more than 90 s showed 99.5% haploid syndrome (Plate 1). These haploids were characterized by a short tail, an enlarged cardiac cavity and a distorted body. The hatched haploid embryos were smaller (1.8–2.1 mm length) and development was also slower than the diploid control (2.8–3.1) (Plate 1). Chromosome preparation from these (UV irradiated) batches showed that the hatchlings were haploid with 29 chromosomes (Plate 2). These haploids were alive till yolk sac absorption.

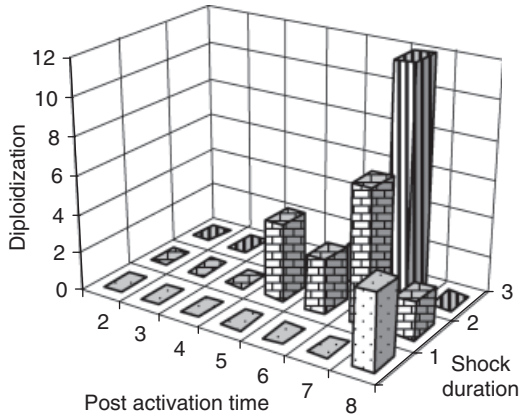

Figure 3 Eggs inseminated with UV-irradiated sperm and heat shocked at 39 °C for different durations of time at different post activation times in minutes.

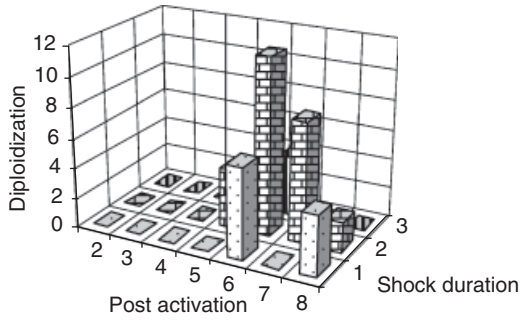

Figure 4 Eggs inseminated with UV-irradiated sperm and heat shocked at 40 °C for different durations of time at different post activation times in minutes.

Diploid gynogenesis

Sperm UV irradiated for 240 s alone were used to activate egg development throughout the diploidization study. There was no significant difference in the fertilization rate between the different groups ($P > 0.05$). The intensity, duration and time of application of the heat shock strongly influenced the production of diploid gynogens (Figs 3–5). Immersion of eggs at 38 °C for different durations failed to induce diploidization (not shown). The eggs heat shocked 7 min after post activation at 39 °C for 3 min resulted in better induction of 12.0% diploid gynogen. When the shock duration was reduced to 2 min at the same embryological age, only 7.0% diploidization was recorded. The shock induction applied at 8-min-post activation for durations of 1 and 2 min yielded only 4% and 2% diploidization respectively (Fig. 3).

The diploidization results achieved at 40 °C are shown in Fig. 4. A maximum of 11.7% diploidization was recorded at 6 min post activation, shocked for 2 min duration, followed by 6.0% and 4.0% diploidization, observed at the same embryological age shocked at 1- and 3-min durations respectively.

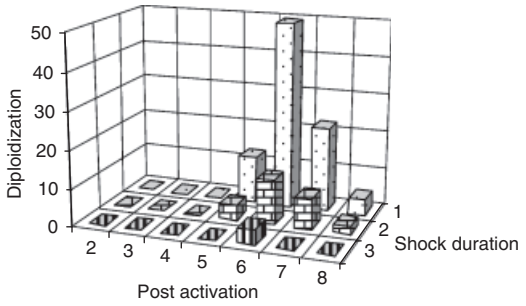

Figure 5 Eggs inseminated with UV-irradiated sperm and heat shocked at 41 °C for different durations of time at different post activation times in minutes.

The shock temperature of 41 °C (Fig. 5) yielded the best and overall maximum of 49.3% at the embryological age of 6 min post activation and a 1-min shock duration. When the shock duration was extended to 2 and 3 min, lower diploidization results of 11.7% and 4.0% were recorded respectively. At the embryological age of 7 min, 22.4% and 8.0% diploidization resulted in the shock durations of 1 and 2 min. The diploid gynogens (Plate 1) had a morphological appearance similar to that of control diploids. Confirmation of diploid gynogens was carried out by a chromosome count ($2n = 58$) (Plate 2). The hatched embryos, in which the diploid condition was not restored, showed a typical haploid morphology (Plate 1). The shock temperature and shock duration were strongly linked. For instance, when shocks were applied at 41 °C, the optimum duration was 1 min (Fig. 5) and at 39 °C; 3 min was the optimum shock duration.

Rearing and sexing

After 6 months of rearing at room temperature 27 ± 1 °C, a total of 113 diploid gynogenetic progenies from seven female fish survived out of 193 fries. The sex ratio in the progenies of diploid gynogens deviated significantly from the expected 1:1 sex ratio (Table 1). In the positive controls and natural controls, the sex ratio was 1:1. Although an approximately 1:1 sex ratio was observed in the control ($P > 0.05$), the sex ratio of the diploid gynogen was biased ($P < 0.05$) towards females. These sex ratio data support the XX/XY sex determination in *H. fossilis*.

Table 1 Sex ratio of diploid gynogen, positive control and natural control in *Heteropneustes fossilis*

Treatment	Maternal parent identification number	Total number fish	Number of males	Number of females	% females	χ^2 (1:1)
Positive control	1	42	18	24	57.14	0.595
	2	38	15	23	63.16	1.289
	3	54	26	28	51.85	0.019
	4	32	16	16	50.00	0.031
	Mean				55.54	
Diploid gynogen	1	14	0	14	100.00	12.071#
	2	23*	0	22	95.65	20.045#
	3	37	0	37	100.00	35.027#
	4	5	0	5	100.00	3.200#
	5	11*	0	10	90.91	8.100#
	6	19	0	19	100.00	17.053#
	7	4	0	4	100.00	2.250#
	Mean				98.23	
Natural control	1	94	42	48	51.06	0.278
	2	89	38	51	57.30	1.618
	3	93	45	48	51.61	0.688
	Mean				57.19	

*One fish undifferentiated.
#$P < 0.05$.

Discussion

Post-irradiation motility of the sperm showed a decline in motility with an increase in UV exposure. Prolonged UV exposure of sperm beyond 270 s might have damaged the sperm, which is reflected in the hatching percentage of embryo (Fig. 2). The UV irradiation of *H. fossilis* sperm resulted in a typical Hertwig curve (Fig. 2). Up to 75 s (first phase) there is a decrease in embryo survival and hatching percentage, and after 75 min, as the irradiation duration of the sperm increases, the survival of embryo also increases. During 120–270 s (second phase), a paradoxical increase in the embryo survival rate was recorded when the irradiation intensity continued to increase. Such typical Hertwig curves were reported for UV irradiation in tilapia, *Oreochromis aureus* (Don & Avtalion 1988) and in *Oryzias latipes* (Ijiri & Egami 1980). As per the explanation of Don and Avtalion (1988), in this paradoxical curve, during the first phase, partial destruction of sperm chromatin had occurred. During the second phase, higher UV irradiation intensifies the destruction of the paternal chromatin completely. Thus, haploid embryos exclusively possessing the maternal genome were formed (Fig. 2). A great advantage of using UV irradiation is that the syngamy of parental pronuclei does not occur, probably due to a massive pyrimidine dimerization that leads to nucleoplasm coagulation of paternal chromatin (Ijiri 1980). Based on the above explanation, in *H. fossilis*, a reliable and easy method for the production of 100% gynogenetic haploid was developed. The UV irradiation for 240 s was adequate for the complete inactivation of sperm genetic material in *H. fossilis*, but retaining its viability for egg activation.

The activated eggs were kept in the dark to prevent photoreactivation of the inactivated chromosomes of sperm and the entire process was carried out under complete darkness as suggested by Van Eenennaam, Van Eenennaam and Medrano (1996) because damaged sperm DNA could be partially photorepaired by exposure to visible light (Thorgaard 1983).

All groups of eggs inseminated with UV-irradiated sperm (above 105 s), without diploidization treatment, developed into 100% abnormal embryos and fry with the characteristic haploid syndrome, showing that even at this low exposure dosage, complete destruction of sperm genetic material had occurred.

The viability of haploid fish embryo at various stages of development seems to be species specific. The haploid embryos of medaka, *O. latipes*, were unable to develop up to the hatching stage (Ijiri & Egami 1980). However, the haploids in *H. fossilis* could survive to the hatching stage and died in 3–4 days, primarily due to exhaustion of the yolk nutrients. The development of the eye and the mouth was totally impaired. Similar results were observed in *C. macrocephalus* (Mongkonpunya, Senawong, Rungsin 1997) in *Oerochromis aurieus* (Don & Avtalion 1988) and in

Tinca tinca (Wang, Liu, Min, Tong, Guan, Han, Gong, Huang, Ren, Zhang & Zheng 2006).

Four different temperature shock trials were used to retain the second polar body. The temperature shock at 38 °C was found to be ineffective in inducing diploidy. Heat shock at 41 °C induced a maximum (49.3%) diploidy when shocked for a 1-min duration and 6 min post activation (Fig. 5). A higher shock temperature (41 °C) was able to retain the second polar body with the best results. The shock temperature of 39 °C resulted in less diploidization and it required a higher shock duration (3 min), and most of the induction occurred during a 3-min shock duration. The shock temperature and duration are the two important parameters that are inversely proportional to each other. This phenomenon is inconsistent with the previous study on *Salmo gairdnei*, which indicated that a heat shock of either 27–30 °C for 10 min or 36 °C for 1 min can be used to induce diploidization (Chourrout 1980). An increased shock temperature and a shorter shock duration itself were sufficient to retain the second polar body. Arai and Wilkins (1987) observed the same phenomenon for retaining the second polar body in brown trout (*Salmo trutta*).

From the present study, it can be observed that the maximum diploidization occurred around 6 min post activation. On comparing all the shock initiation times after activation, durations of 5, 6 and 7 min were found to be more sensitive to thermal shock, resulting in diploidization, and in other words, the time from 2 to 5 min and after 8 min could be related to the increase in tolerance of the second polar body to thermal shock, and the eggs developed as haploid fry. Thus, comparison of this study with other species such as *C. gariepinus* (Varadi *et al.* 1999); *Oreochromis niloticus* (Mair 1993); and *P. gonionotus* (Pongthana *et al.* 1995) indicates that an effective shock temperature with a wide range and optimum shock duration for a particular shock temperature must be determined for each species. It can be summarized that the heat shock was effective in preventing the extrusion of the second polar body, favouring diploid gynogens in *H. fossilis*, with the maximum yield of 49.3% diploid gynogens.

Gynogenetic offspring of several fish species have lower survival rates than normal controls (Chourrout & Quillet 1982). In the present study (180 days), the post-hatch survival of gynogenetic *H. fossilis* was lower than that of their positive controls (Table 1). This might be due to the expression of harmful recessive genes resulting from the high degree of inbreeding. Nakanishi (1987) found a relatively high degree of homozygosity, in the first and second generation of gynogenetic diploids produced by retaining the second polar body in goldfish, *Carassius auratus* L. Therefore, the lower survival of the gynogenetic diploids in Indian catfish could be related to the higher degree of homozygosity of harmful genes derived from their female parent. We suggest that low frequencies of recombination occur in this species and cause a rapid increase in the inbreeding level. This is followed by the expression of lethal and defective genes. The degree of expression of these genes also varies in each maternal parent and genetic factor.

A variety of genetic sex-determination systems have been recorded in fish (i.e. XX, ZW and XO) unlike mammals and birds (Yamasaki 1983). Sex determination in fish is controlled by complex mechanisms. A number of external factors namely, temperature (Strussman, Choon, Takashima & Oshiro 1993) social behaviour (Shapiro 1987) and surgical removal of the gonads (Lowe & Larkin 1975) are even known to induce sex reversal in fish, in which the process of sex determination and differentiation is still more complex. Sex chromosomes of fish develop from purely polygenic controls, to those with dominant sex-determination factors mixed with autosomal controls, to highly evolved sex chromosomes with heterogametic (XY) males or heterogametic (ZW) females (Devlin & Nagahama 2002). In *H. fossilis*, all female gynogens obtained in the study suggest a female homogametic (XX/XY) type of sex determination. A similar conclusion on the female homogametic nature has been drawn in walking catfish, *C. macrocephalus* (Na-Nakorn 1995), channel catfish, *I. punctatus* (Davis, Simco, Goudie, Parker & Cauldwell 1990), Atlantic halibut, *H. hippoglossus* (Tvedt *et al.* 2006), and goldfish, *C. auratus* (Oshiro 1987). Further studies are needed to observe the influence of external factors on sex determination.

Acknowledgments

This work was funded by the Indian Council of Agricultural Research, Govt. of India. The first author would like to especially thank Ms. Rosalie Shaffer, Information Specialist, NMFS Library, USA, for providing the necessary reference materials.

References

Arai K. (2001) Genetic improvement of aquaculture finfish species by chromosome manipulation techniques in Japan. *Aquaculture* **197**, 205–228.

Arai K. & Wilkins N.P. (1987) Triploidization of brown trout (*Salmo trutta*) by heat shock. *Aquaculture* **64**, 97–103.

Bye B.J. & Lincoln R.F. (1986) Commercial method of sexual maturation in rainbow trout (*Salmo gairdneri* R.). *Aquaculture* **57**, 299–292.

Benfey T.J. (1996) Use of all-female and triploid salmonids for aquaculture in Canada. *Bulletin of the Aquaculture Association of Canada* **2**, 6–8.

Chourrout D. (1980) Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (*Salmo gairdneri*). *Reproductive Nutrition Development* **20**, 727–733.

Chourrout D. & Quillet E. (1982) Induced gynogenesis in the rainbow trout: sex and survival of production of all triploid populations. *Theoretical Applied Genetics* **63**, 201–205.

Christopher J.G. (2001) *Genetic manipulation studies in the Indian catfish Heteropneustes fossilis*. PhD thesis, Submitted to Manonmaniam Sundaranar University, Tirunelveli, India, 196pp.

Christopher J.G., Murugesan A.G. & Sukumaran N. (2009) Genetic inactivation of stinging catfish (*Heteropneustes fossilis*) sperm with UV irradiation. *Journal of Applied Aquaculture* **21**, 1–10.

Davis K.B., Simco B.A., Goudie C.A., Parker N.C. & Cauldwell W. (1990) Hormonal sex manipulation and evidence for female homogamety in channel catfish. *General and Comparative Endocrinology* **78**, 218–223.

Dehadrai P.V., Yusuf K.M. & Das K.S. (1985) Package of practices for increasing production of air breathing fishes. In: *Aquaculture Extension Manual (ICAR). New series, No. 3* (ed. by Das K.S.) pp. 1–4. ICAR Press, Barrackpore, India.

Devlin R.H. & Nagahama Y. (2002) Sex determination in fish: overview of genetic, physiology and environmental influences. *Aquaculture* **208**, 191–364.

Don J. & Avtalion R.R. (1988) Production of F_1 and F_2 diploid gynogenetic tilapias and analysis of the "Hertwig curve" obtained using ultraviolet irradiated sperm. *Theoretical and Applied Genetics* **76**, 253–259.

Gheyas A.A., Mollah M.F.A. & Hussain M.G. (2001) Triploidy induction in stinging catfish *Heteropneustes fossilis* using cold shock. *Asian Fisheries Science* **14**, 323–332.

Goudie A.C., Simco A.B., Davis B.K. & Liu Q. (1995) Production of gynogenetic and polyploid catfish by pressure induced chromosome set manipulation. *Aquaculture* **133**, 185–198.

Hulata G. (2001) Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. *Genetica* **111**, 155–173.

Ihssen P.E., Mckay L.R., McMillan I. & Philips R.B. (1990) Ploidy manipulation and gynogenesis in fishes. *Transactions of the American Fisheries Society* **119**, 698–717.

Ijiri K. (1980) Gamma-ray irradiation of the sperm of the fish *Oryzias fates* and induction of gynogenesis. *Journal of Radiation Research* **21**, 263–270.

Ijiri K. & Egami N. (1980) Hertwig effect causes by UV-irradiation of sperm of *Oryzias fates* (Teleost) and its photoreactivation. *Mutation Research* **69**, 241–248.

Kaastrup P.K. & Horlyck V. (1987) Development of single method to optimize the conditions for producing gynogenetic offspring, kusing albing rainbow trout I Richardson, k females as indicator for gynogenesis. *Journal of Fish Biology* **31**, 29–33.

Kawamura K. (1998) Sex determination system of the rosy bitterling, *Rhodeus ocellatus ocellatus*. *Environmental Biology of Fishes* **52**, 251–260.

Kligerman A.D. & Bloom S.E. (1977) Rapid chromosome preparations from solid tissue of fishes. *Journal of Fisheries Research Board Canada* **34**, 266–269.

Komen H. & Thorgaard G.H. (2007) Androgenesis, gynogenesis and the production of clones in fishes: a review. *Aquaculture* **269**, 150–173.

Lal K.K., Barman A.S., Punia P., Khare P., Mohindra V., Lal B., Gopalakrishnan A., Sah R.S. & Lakra W.S. (2009) Effect of extender composition on sperm cryopreservation of Asian catfish *Heteropneustes fossilis* (Bloch) and *Clarias batrachus* (Linnaeus). *Asian Fisheries Science* **22**, 137–142.

Lowe T.P. & Larkin J.R. (1975) Sex reversal in *Betta splendens* (Regan) with emphasis on the problem of sex determination. *Journal of Experimental Zoology* **191**, 25–32.

Mair G.C. (1993) Chromosome manipulation in tilapia techniques problems and prospects. *Aquaculture* **111**, 227–244.

Mirza J.A. & Shelton W.L. (1988) Induced gynogenesis and sex reversal in silver carp. *Aquaculture* **68**, 1–14.

Mongkonpunya K., Senawong C. & Rungsin W. (1997) Morphological, chromosome counts and genomic analysis of abnormal haploid offspring of clariids (*Clarias macrocephualus*). *Thailand Journal of Agriculture Science* **30**, 423–433.

Nagy A., Bercsenyi M. & Csanyi V. (1981) Sex reversal in carp (*Cyprinus carpio*) by oral administration of methyltestosterone. *Journal of Fisheries Research Board Canada* **38**, 725–728.

Nakanishi T. (1987) Histocompatibility analyses in tetraploids induced from clonal triploid crucian carp and in gynogenetic diploid goldfish. *Journal of Fish Biology* **31** (Suppl. A), 35–40.

Na-Nakorn U. (1995) Comparison of cold and heat shocks to induce diploid gynogenesis in Thai walking catfish (*Clarias macrocephalus*) and performances of gynogens. *Aquatic Living Resources* **8**, 333–341.

Na-Nakorn U., Rangsin W. & Asunkul S.W (1993) Suitable conditions for inducing of gynogenesis in the catfish *Clarias macrocephalus*; using sperm of *Pangasius sutchi*. *Aquaculture* **118**, 53–62.

Oshiro T. (1987) Cytological studies on diploid gynogenesis induced in the loach *Misgurnus anguillicaudatus*. *Bulletin of Journal Society Science Fish* **53**, 933–942.

Pandian T.J. & Koteeswaran R. (1998) Ploidy induction and sex control in fish. *Hydrobiologia* **384**, 167–243.

Pongthana N., Penman D.J. & Karnasuta J. (1995) Induced gynogenesis in the silver barb (*Puntius goniontus* Bleeker) and evidence for female homogamety. *Aquaculture* **135**, 267–276.

Purdom C.E. (1969) Radiation induced gynogenesis and androgenesis in fish. *Heredity* **24**, 431–444.

Purdom C.E. (1983) Genetic engineering by the manipulation of chromosome. *Aquaculture* **33**, 287–300.

Purdom C.E. & Lincoln R.F. (1973) Chromosome manipulation in fish. In: *Genetics and Mutagenesis of Fish* (ed. by J. Schroder), pp. 83–39. Springer, Berlin, Germany.

Quillet E., Foisil L., Chevassus B., Chourrout D. & Liu F.G. (1991) Production of all-triploid and all-female brown trout for aquaculture. *Aquatic Living Resource* **4**, 27–32.

Rexlin S. (1996) *Effect of different cryoprotectants on the longevity of sperm life in Tilapia Oreochromis mosambicus and the Indian catfish Heteropneustes fossilis*. Dissertation, Manonmaniam Sundaranar University, Tirunelveli, India.

Saxena P.K. & Sandhu R. (1993) Influence of temperature on ovarian recrudescence in the Indian catfish, *Heteropneustes fossilis*. *Journal of Fish Biology* **44**, 168–171.

Shapiro D.Y. (1987) Differentiation and evolution of sex change in fishes. *Bio Science* **37**, 490–497.

Stanley J.G. (1976) Female homogamety in grass carp (*Ctenopharhyngodon idella*) determined by gynogenesis. *Journal of Fisheries Research Board Canada* **33**, 1372–1734.

Strussman C.A., Choon B.Ng., Takashima F. & Oshiro T. (1993) Triploidy induction in an atherinid fish, the pejerry (*Odontesthes bonariensis*). *Progressive Fish Culturist* **55**, 83–89.

Thorgaard G.H. (1983) Chromosome set manipulation and sex control in fish. In: *Fish Physiology, Vol IX B* (ed. by W.S. Hoar, D.J. Randall & E.M. Donaldson), pp. 405–434. Academic Press, New York, NY, USA.

Thorgaard G.H. & Allen S.K. (1987) Chromosome manipulation and markers in fishery management. In: *Population Genetics and Fishery Management* (ed. by N. Ryman & F.M. Utter), pp. 319–331. University of Washington Press, Seattle, WA, USA.

Thorgaard G.H., Allendorf F.W. & Knudsen K.L. (1983) Gene-centromere mapping in rainbow trout: high interference over long map distances. *Genetics* **103**, 771–783.

Tiwary B.K., Kirubagaran R. & Ray A.K. (1997) Induction of triploidy by cold shock in catfish, *Heteropneustes fossilis* (Bloch). *Asian Fisheries Science* **10**, 123–129.

Tiwary B.K., Kirubagaran R. & Ray A.K. (2000) Gonadal development in triploid *Heteropneustes fossilis*. *Journal of Fish Biology* **57**, 1343–1348.

Tvedt H.B., Benfey T.J., Martin-Robichaud D.J., McGowan C. & Reith M. (2006) Gynogenesis and sex determination in Atlantic Halibut (*Hippoglossus hippoglossus*). *Aquaculture* **10**, 573–583.

Van Eenennaam A.L., Van Eenennaam J.P. & Medrano J.P. (1996) Rapid verification of meiotic gynogenesis and polyploidy in white sturgeon (*Acipenser transmontanus* Richardson). *Aquaculture* **147**, 177–189.

Varadaraj K. (1993) Production of viable haploid *Oreochromis mossambicus* gynogens using UV irradiated sperm. *Journal of Experimental Zoology* **167**, 460–467.

Varadi L., Benko I. & Varga J. (1999) Induction of diploid gynogenesis using interspecific sperm and production of tetraploids in African catfish *Clarias gariepinus* Burchell (1822). *Aquaculture* **173**, 401–411.

Volckaert F.A.M., Galbusera P.H.A., Hellemant B.A.S., Van den Heute C., Vanstaen D. & Ollevier F. (1994) Gynogenesis in the African catfish (*Clarias gariepinus*). 1: induction of meiogynogenesis with thermal and pressure shocks. *Aquaculture* **128**, 221–233.

Wang J., Liu H., Min W., Tong J., Guan M., Han Y., Gong L., Huang Z., Ren J., Zhang J. & Zheng H. (2006) Induced meiotic gynogenesis in tench, *Tinca tinca* (L.) using irradiated heterogenic sperm. *Aquaculture International* **14**, 35–42.

Yamasaki F. (1983) Sex control and manipulation in fish. *Aquaculture* **33**, 329–354.

You C., Yu X., Tan D. & Tong J. (2008) Gynogenesis and sex determination in large-scale loach *Paramisgurnus dabryanus* (Sauvage). *Aquaculture International* **16**, 203–214.

Zar J.H. (1984) *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, NJ, USA. 718 pp.