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Comparison of cold and heat shocks to induce diploid gynogenesis in Thai walking catfish (*Clarias macrocephalus*) and performances of gynogens

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Abstract

Large numbers of female broodstock *(Clarias macrocephalus)* are required each year to produce hybrid catfish which retain the maternal meat quality of Thai walking catfish and the paternal fast growing and disease resistant traits of the African catfish (*Clarias gariepinus*). Therefore, diploid meiotic gynogens were induced in Thai walking catfish and their potential use for aquaculture was evaluated. Induction was performed using UV-irradiated sperm of stripped catfish (*Pangasius sutchi*) followed by either cold or heat shock at 4.5 min after activation. Heat shock at 42 °C for 1 min was the best among various heat shock regimes. Cold shock (7 °C for 14 min) gave significantly higher percentages of meiotic diploid fry (26-41 %) than the heat shock (12-26 %). Six-month-old meiotic gynogens were all female and showed slightly inferior growth to male and female controls. The six-month-old control-males and females and the gynogens were not different in size. Survival of the gynogens (73 ± 8 %) was slightly inferior to the mixed sex control (87 ± 10 %). Gonado-somatic index of gynogens at 1 year of age (5.7 %) was slightly inferior to that of the control female (8.8 %). Therefore, the direct use of all female meiotic gynogens to enhance production of Thai walking catfish, may be doubtful. However, their use for breeding purposes is promising providing that inbreeding depression is eliminated.

Keywords: Fish culture, polyploids, temperature shock, *Clarias macrocephalus*, gynogenesis, irradiation.

Comparaison de chocs thermiques pour l'induction d'une gynogenèse diploïde chez le poisson-chat Clarias macrocephalus.

Résumé

Des stocks importants de femelles *Clarias macrocephalus* sont nécessaires chaque année pour produire des poissons-chats hybrides qui détiennent à la fois la qualité de la chair maternelle, et la croissance rapide et la résistance aux maladies paternelles du poisson-chat africain *Clarias gariepinus*. Par conséquent, des individus gynogénétiques diploïdes ont été induits chez le poisson-chat *Clarias macrocephalus* et leurs potentialités ont été évaluées pour l'aquaculture. L'induction a été réalisée par irradiation UV du sperme de poisson-chat *Pangasius sutchi* suivie de chocs thermiques soit chauds soit froids 4,5 min après l'activation. Parmi les chocs chauds, ceux effectués à 42 °C durant 1 min étaient les plus performants. Les chocs froids (7 °C pendant 14 min) ont donné de plus forts pourcentages significatifs de larves diploïdes (26-41 %) que les chocs chauds (12-26 %). Les individus gynogénétiques âgés de 6 mois étaient tous femelles et présentaient des croissances légèrement inférieures aux témoins mâles et aux témoins femelles. Les témoins mâles, les témoins femelles et les individus gynogénétiques âgés de 6 mois ne présentaient pas de différence de taille. Le taux de survie des individus gynogénétiques (73 ± 8 %) était légèrement inférieur à ceux des témoins des deux sexes (87 ± 10 %). L'indice gonado-somatique des individus gynogénétiques âgés de 1 an (5,7 %) était légèrement inférieur à celui des femelles témoins (8,8 %). Par conséquent, l'usage direct de femelles gynogénétiques pour améliorer la production de poissons-chats de Thaïlande est incertain. Cependant, leur utilité à des fins de reproduction est prometteur.

Mots-clés : Élevage de poissons, polyploïdie, choc thermique, irradiation, *Clarias macrocephalus.*

INTRODUCTION

Culture of *Clarias* sp. in Thailand, although established for more than 40 years, has been limited by the slow growth rate and disease susceptibility of the local species, *C. batrachus* and *C. macrocephalus* under culture conditions (Diana *et al.*, 1985).

The problems have been overcome since the introduction of the hybrid between female *C. macrocephalus* and male *C. gariepinus* (African catfish) in 1990 (Nukwan *et al.*, 1990).

Because of its desirable meat quality retained from the maternal species, improved growth rate and disease resistance contributed from the paternal genome, the hybrid has replaced the local species and led to at least a 50% increase in *Clarias* culture area in the country (Dept. of Fisheries, 1993).

This has resulted in a shortage of female Thai walking catfish, not only needed for breeding purposes, but also for consumption since the mature ovaries are a popular food among Thais.

Induction of gynogenesis is a promising technique to produce all-female stocks in a female homogametic species (Stanley, 1976; Nagy *et al.*, 1978; Refstie *et al.*, 1982). In Thai walking catfish gynogenesis has been induced using UV-irradiated sperm of stripped catfish (*Pangasius sutchi*) and diplodized by subjecting activated eggs to cold shock (Na-Nakorn *et al.*, 1993*b*) and diploid yield of approximately 60% with the total hatching rate of 70% of the control were obtained. However, results of the treatment fluctuated over different egg batches.

It was previously believed that cold shock is more efficient for induction of polyploidy in warm-water fishes (Linhart *et al.*, 1991). It was partly confirmed in species such as grass carp, *Ctenopharyngodon idella* (Cassani and Caton, 1985), Nile tilapia, *Oreochromis niloticus* (Don and Avtalion, 1988) and channel catfish, *Ictalurus punctatus* (Wolters *et al.*, 1981; Bidwell *et al.*, 1985). However, heat shock was also effective in inducing triploidy in *Oreochromis aureus* (Don and Avtalion, 1988), *O. mossambicus* (Pandian and Varadaraj, 1988), common carp, *Cyprinus carpio* (Recoubratsky *et al.*, 1992) and Siamese fighting fish, *Betta splendens* (Kavumpurath and Pandian, 1992). Moreover, a comprehensive comparison made by Hussian *et al.* (1991) indicated that higher and more consistent yield of triploid Nile tilapia was achieved from heat shock rather than cold shock.

Although retention of the second polar body was successfully induced by cold shock in species of *Clarias*; such as *C. gariepinus* (Henken *et al.*, 1987), *C. batrachus* (Manickam, 1991) and *C. macrocephalus* (Na-Nakorn and Lekaanantakul, 1993; Na-Nakorn *et al.*, 1993*b*) the efficiency of heat shock in this species has not been studied.

Therefore, attempts were made in this study to compare the efficiency of cold shock and heat shock in inducing diploid meiotic gynogenesis in *C. macrocephalus*. More consistent results in different egg batches and a higher hatching percentage were expected in the heat-shock experiment which is probably less harmful than cold shock due to a much shorter shock duration needed.

MATERIALS AND METHODS

Sperm collection and irradiation

Sperm used for induction of gynogenesis was collected from male stripped catfish which were injected with 200 IU HCG/kg-bw 4 hours prior to stripping. Sperm were diluted in Ringer's solution to make a dilution of 1:100 (sperm: Ringer's solution). UV irradiation was performed following Na-Nakorn *et al.* (1993*b*); the diluted sperm were poured into a Petri dish to make a layer of less than 2 mm. Irradiation was performed by placing the diluted sperm 30 cm, below a 30 watt UV germicidal tube. Irradiation lasted 2 minutes.

Sperm of Thai walking catfish was used to fertilize eggs of control groups in experiments on induction of gynogenesis as well as an experiment on evaluation of gonadal maturation of gynogens. Male fish were injected with 200 IU HCG/kg-body weight 6 hours prior to sperm collection when whole testes were removed and ground. Dilution of approximately 1:100 sperm to Ringer's solution was made.

Egg collection

Gravid females of Thai walking catfish, selected for their swelling and soft bellies and protruding-pinkish urogenital papillae, were injected with 3,000 IU HCG/kg-bw 12 hours prior to stripping.

Incubation

Incubation was conducted in plastic bowls of 50 cm in diameter, 15 cm deep with water flow-through system. A fine mesh nylon net was fixed at 10 cm above bottom of each bowl. Eggs were spread evenly on this nylon net.

Identification of fry

Fry were identified into 3 types: haploid, diploid gynogens and hybrid. Diploid gynogens were recognized by their long tails and normal appearance while haploid individuals had a thick short body and curved tail. Hybrid larvae, when they occurred, were characterized by a long tail, a small head and a small yolk sac as well as a bigger cavity between the yolk sac and the anterior part of the body compared to diploid larvae (Na-Nakorn *et al.*, 1993*b*). The fry obtained from every treatment group were kept for at least a week when the hybrid could be identified more efficiently.

Although hybrids had not been produced in previous experiments on induction of gynogenesis in *C. macrocephalus* using irradiated sperm of *P. sutchi* (Na-Nakorn *et al.*, 1993*b*) their distinguishable characters were reported in another hybridization experiment (Na-Nakorn *et al.*, 1993*a*). They were easily identified within a week after hatching due to their appearances as well as their swimming behaviour (Na-Nakorn, unpubl. data).

Data analysis

A completely randomized design was used to analyse the influence of temperature and shock durations for heat shock. Data obtained from a comparison of heat and cold shock and an experiment on evaluation of performances of meiotic gynogens were analysed using a randomized complete-block design. Means were compared using Duncan's New multiple range test (Steel and Torrie, 1980). Percentages were transformed using arcsin transformation prior to analysis.

Experiment on suitable temperature and shock durations for heat shock

Heat shock was conducted using temperature of 35, 37, 40 and 42 °C with shock durations of 1-7 min with 1 min interval. Since only two temperature-controlled basins were available, different combinations of 2 shock temperatures were used to induce gynogenesis of eggs from a single female at a time and data were pooled, which resulted in 3 replicates. Approximate 200 eggs obtained from a single female were mixed with 0.5 ml of irradiated sperm and put into each of 28 9-square inch fine mesh cages. The cage were immediately dipped into a tank containing water at ambient temperature (27-29 °C) when the time was considered 0 minute after activation. At 4.5 min after activation (Na-Nakorn *et al.*, 1993*b*) they were transferred to heat shock basins. At every 1 minute two cages were removed from each shock-basin and eggs were transferred to hatching bowls resulted in 7 shock durations. Every time gynogenesis induced controls were generated by mixing 2 portions of eggs (200 each) with sperm of its own species. Incubation was performed in hatching bowls under ambient temperature throughout the experiment. Hatching commenced 30 hours later when fry were identified as haploid, diploid gynogens or hybrid. Hatching percentage and percentage of diploid gynogens were estimated based on total number of eggs incubated. Data obtained from each 2 cages subjected to the same treatment were averaged and presented as values relative to those of the respective control.

Comparison was made between results of the best shock duration for each temperature at which analysis was previously made.

Comparison between heat-shock and cold-shock

The best condition of heat shock, 42 °C for 1 min, was compared to cold shock, 7 °C for 14 min (Na-Nakorn *et al.*, 1993*b*).

Eggs of 11 females were separately collected and divided into 2 portions. Each portion was mixed with irradiated sperm of striped catfish and subjected to cold shock (7 °C for 14 min) or heat shock (42 °C for 1 min) starting at 4.5 min after activation. After the shock, approximately 200 eggs from each portion were incubated in each of 4 hatching bowls.

After hatching, larvae were counted and identified into 3 types: haploid, diploid gynogens and hybrids. Total hatching percentage and percentage of diploid gynogens were estimated based on total number of eggs in each bowl. Data from each set of 4 bowls were averaged.

Evaluation of performance of meiotic gynogens

Mass production of meiotic gynogens was effected by cold shock (Na-Nakorn *et al.*, 1993*b*). Eggs collected from each of 3 females were divided into 2 portions which were either fertilized with sperm of its own species without cold shock or mixed with UV-irradiated sperm of striped catfish followed by cold shock. Larvae obtained from each group were separately raised for 3 weeks when they reached a size of approximately 1 inch (0.14 g). The fingerlings from each group were stocked in each of 6 ponds of 1.5×2 m at a stocking density of 100 fingerlings/pond. Initial sizes of fish stocked in each pond were not statistically different.

They were fed *ad libitum* with a 30% protein commercially available pelleted feed twice a day. Twenty per cent of water content was changed daily. After 21 weeks of rearing the fish were harvested. Measurement was made for weight and length and survival rates were estimated. Data were analysed using a randomized complete block design (Steel and Torrie, 1980).

After harvesting fish were branded according to each female parent using hot-wire. The branded control fish and gynogens were separately stocked in each of 1.5×2 m cages fixed in an earthenware pond in order to provide suitable environment for gonadal development. This resulted in different stocking rates of 219 gynogens/cage and 226 control fish/cage. Moreover, some of the small fish died owing to the branding technique, therefore growth and survival rate to 1 year of age were not investigated.

Identification of sexual types

Male and female Thai walking catfish can be clearly identified based on the shape of their urogenital papillae. The urogenital papilla of the male is a slender oval with a pointed tip while that of a female is quite

round with a blunt tip. Fish that could not be identified were killed for direct examination of the gonad.

Evaluation of gonadal development

At 1 year of age experimental fish were harvested. Two replicates of fifteen fish were randomly taken from each group for induced ovulation and GSI estimation.

Estimation of gonadosomatic index (GSI)

Data on individual body weight and ovary weight were collected in 15 fish randomly taken from each group and GSI was calculated.

Evaluation of ovary maturation

Fifteen fish randomly taken from each group were injected with 30 ug/kg-bw LHRHa (Suprefact) plus 5 mg/kg-bw domperidone (Motilium). Injected fish were stripped 16 hours after injection.

Number of eggs/gram, which roughly represented size of eggs, was determined in each female. The data was averaged within each group and was used to calculate the total number of eggs obtained from each female. Regression equations of fecundity on body weight was determined for the control fish and gynogens (Steel and Torrie, 1980). Samples of about 400 eggs each were taken from each female and fertilized with mixed sperm obtained from 3 males of the same species. After 30 hours of incubation (ambient temperature of 27-29 °C) when hatching commenced, hatching rate was determined.

RESULTS

Suitable temperature and shock duration for heat shock

Mean hatching percentages and percentages of diploid larvae are shown in *table* 1. Heat shock at 35° and 37 °C, regardless of shock duration, reduced hatching rate of the heat-shocked groups to 15.5-28.3 % and 18.4-32.4 % of the normally fertilized ones respectively. Almost all of the gynogenetic larvae were haploid.

In egg groups subjected to 40° and 42 °C heat shock, the hatching rates decreased dramatically when shock duration increased. However, trends for percentages of diploid fry obtained from the two temperatures were different. At a shock duration of 1 min a low percentage of diploids was observed in the group subjected to temperature of 40 °C. However, when shock durations were increased to 2 and 3 min more diploid larvae were produced. Heat shock (40 °C) applied for 4 min resulted in a severe decrease in both hatching rate and percentage of diploid larvae. Heat shock at 42 °C for 1 min yielded the highest hatching percentage and percentage of diploid gynogens. A shock duration of 2 min significantly lowered the hatching percentage and percentage of diploid larvae which, however, were higher than those of the groups subjected to longer shock durations. Temperatures of 40 and 42 °C were completely lethal to Thai walking catfish eggs when they were applied for more than 5 and 3 min respectively.

Since no significant differences were shown among the eggs subjected to different shock durations in both 35° and 37 °C groups, a shock regime was drawn

Table 1. – Mean hatching percentages and percentages of diploid larvae obtained from induced gynogenesis of Thai walking catfish eggs using UV-irradiated sperm of stripped catfish followed by different degrees of heat shock (35-42 °C) with shock durations of 1-7 min initiated 4.5 min after water activation of eggs. Percentages are presented relatively to control.

Shock duration (min)	Temperature (°C)			
	35	37	40	42
		Hatching percentage		
1	21.0±7.2 a	18.4±11.3 a	23.9±9.8 a	43.7±7.3 a
2	28.3±19.0 a	20.7±11.4 a	21.6±12.6 a	11.0±7.1 c
3	17.9±8.0 a	28.6±6.2 a	17.5±8.0 a	1.3±1.3 c
4	23.2±10.3 a	23.2±9.6 a	4.9±5.0 b	0 c
5	15.5±6.5 a	32.4±14.0 a	0 c	0 c
6	22.5±9.1 a	25.4±10.9 a	0 c	0 c
7	18.9±7.1 a	31.5±17.7 a	0 c	0 c
		Percentage of diploid larvae		
1	1.9±1.2 a	1.8±0.8 a	1.2±1.2 b	21.8±7.3 a
2	1.1±1.0 a	0.6±1.0 a	9.6±4.7 a	4.4±2.0 b
3	0.3±0.3 a	0.4±0.4 a	10.9±5.5 a	0.4±0.5 c
4	1.0±0.7 a	1.2±1.0 a	1.6±1.0 b	0 c
5	0.7±0.5 a	0.7±0.5 a	0 b	0 c
6	1.3±0.8 a	0.7±0.5 a	0 b	0 c
7	0.8±0.6 a	0.2±0.2 a	0 b	0 c

Note: Means in the same column followed by different letters are significantly different ($p<0.05$).

from each temperature-group based on the highest percentage of diploid larvae with a smaller standard deviation. They were 6 minutes in the 35 °C-group and 1 min in the 37 °C-group. In the 40 and 42 °C groups the best shock durations were 3 min at 40 °C and 1 minute at 42 °C.

A comparison made between the four heat shock regimes showed that heat shock at 42 °C for 1 min gave the highest diploid percentage followed by the 40 °C-3 min groups, whereas heat shock of 35 °C for 6 min and 37 °C for 1 min gave very low percentages of diploid larvae (*table* 2).

Table 2. – Mean percentages (±SD) of diploid larvae obtained from induced gynogenesis of Thai walking catfish eggs using irradiated sperm of stripped catfish followed by heat shock (initiated 4.5 minutes after activation). Only a shock duration that yielded the best percentage of diploid larvae was taken from each shock temperature and a comparison was made among those at the 4 temperature levels (35-42 °C).

Temperature and shock durations (°C-min)	Hatching (%)		Diploid larvae (%)	
35 °C-6 min	22.5±9.1	b	1.3±0.8	c
37 °C-1 min	18.4±11.3	b	1.8±0.8	c
40 °C-3 min	17.5±8.0	b	10.9±5.5	b
42 °C-1 min	43.7±7.3	a	21.8±7.3	a

Note: Means in the same column followed by different letters are significantly different ($p<0.05$).

A comparison between heat shock and cold shock

Differences between females were observed ($p<0.01$) both for hatching percentages and percentage of diploid larvae. Eggs subjected to cold shock gave higher mean hatching rates (36.8±8.5%) and mean percentages of diploid larvae (29.6±6.7%) than those subjected to heat shock which yielded mean hatching rates of 19.5±8.1% and mean percentages of diploid gynogens of 10.5±5.1% ($p<0.01$) (*table* 3).

Table 3. – Mean hatching percentages (±SD) and percentages of diploid larvae (±SD) obtained from induced gynogenesis in eggs obtained from 11 Thai walking catfish females using UV-irradiated sperm of stripped catfish. Diploidization was induced using cold shock (7 °C, 14 minutes) or heat shock (42 °C, 1 min) initiated 4.5 min after activation.

Female no. [1]	Hatching (%)[2]		Dilpoid larvae (%)[3]	
	7 °C	42 °C	7 °C	42 °C
1	29.8±5.2	24.4±16.9	19.7±4.1	5.8±0.9
2	32.8±3.7	22.0±2.6	27.5±3.9	7.1±2.7
3	36.3±4.8	9.5±2.6	33.8±4.2	4.7±0.9
4	49.3±4.7	4.1±1.6	41.1±3.5	3.8±1.2
5	36.1±3.5	22.1±2.9	34.3±3.5	19.9±2.1
6	42.6±2.2	35.7±3.1	35.3±2.4	17.2±3.8
7	38.1±4.2	11.7±4.5	32.8±3.5	8.3±3.1
8	38.0±4.3	20.4±6.5	29.0±3.1	10.6±2.5
9	52.4±4.8	22.1±2.4	31.3±3.0	12.7±2.4
10	24.9±2.7	18.3±3.2	20.7±3.9	16.2±3.4
11	24.6±3.6	23.9±2.9	19.8±3.0	8.9±2.1
Mean	36.8±8.5	19.5±8.1	29.6±6.7	10.5±5.1

([1]) Differences between females are significant ($p<0.01$).
([2]) Differences are significant between mean hatching rates of the 7 °C and 42 °C-groups as well as between mean percentage of diploid larvae of the 7 °C and 42 °C-groups.

Growth, survival rate and sexual types

At six months of age experimental fish were smaller than those under normal culture conditions which were approximately 100 g (Na-Nakorn, 1994). Non-significant differences were shown between sizes of control male, female and gynogens. The control females were 45.4±7.0 g; 17.5±0.9 cm while male fish of the same group were slightly smaller (40.3±8.5 g; 17.1±1.1 cm) than females, although statistical analysis showed no significant difference ($p>0.05$). Meiotic gynogens were 39.5±15.2 g in weight and 16.1±1.8 cm in length (*table* 4).

The survival rate of gynogens (7300±8.3%) was slightly lower than the mixed-sex control (87.3±10.1%) although the difference was not significant.

Two hundred and nineteen gynogenetic fish (from 3 females) were all female while sex ratios (male: female) of the 3 control groups were 1:1.4 (total of 98), 1:1.3 (total of 95) and 1:0.7 (total of 73) and were not statistically different from 1:1 ratio.

Table 4. – Mean body weight (±SD), length (±SD), and survival rate (±SD) at 6 months of age and GSI at 1 year of age of gynogenetic Thai walking catfish and the control male and females.

	BW (±SD) (g)		BL (±SD) (cm)		Survival rate (±SD) (of both sexes) (%)		GSI (of female)	
Control (female)	45.4±7.0	a	17.5±0.9	a	87.3±10.1	a	8.8±1.4	a
Control (male)	40.3±8.5	a	17.1±1.1	a		a		a
Gynogens (all female)	39.5±15.2	a	16.1±1.8	a	73.0±8.3	a	5.7±1.7	a

Note: Means in the same column follow by the same letters are not significantly different ($p>0.05$).

Gonadosomatic index (GSI)

GSI, investigated at 1 year of age, of the gynogens varied considerably (ranged from 2.2-9.0%) while differences among those of the control groups were small (GSI ranged 6.6-10.4%). The mean GSI of gynogens (5.7±1.7%) was slightly lower than that of the female control (8.8±1.4%) although differences were not significant ($p>0.05$) (*table* 4). However, the colour of ovaries was quite similar in both groups. They were deep brown which indicated the presence of mostly mature oocytes.

Gonadal maturation

At 1 year of age, when breeding trials were conducted, body weight of the control group and the gynogens ranged between 45-150 g and 40-190 g respectively. Ovulation response was determined at 16 hours after injection, when injected fish were gently stripped. The results are shown in *table* 5. All fish in the control group could be stripped while only 40 of 45 injected gynogens yielded ripe eggs.

Egg size and fecundity

Number of eggs/gram (*table* 5) varied considerably in both groups. This was probably because of different amounts of ovarian fluid present in the egg mass rather than different sizes of eggs. Therefore, a conclusion about a comparison of egg sizes could not be made.

The regression equation for number of eggs on body weight of the control group based on data obtained from 45 females of 45-150 g was

$$F = -1\,015 + 49.0\,W$$

and that of the gynogens (based on data from 40 females sized 40-190 g) was

$$F = -2\,263.4 + 51.3\,W$$

Therefore the estimated number of eggs for a 100 g fish were 3886 and 2872 eggs in the control and gynogens respectively (*table* 5). The result revealed that the gynogens tended to have fewer eggs/female than the respective control.

Table 5. – Number of ovulated female fish after injection of LHRHa + domperidone (30 µg/kg BW + 5 mg/kg BW), number of eggs/g-eggs, estimated number of eggs/100 g-BW and hatching rate of control and gynogenetic females.

Ovulated females/total	No. of eggs/ g-eggs	No. of eggs/* 100 g-bw	Hatching (%)
Control 45/45	675.1±63.8	3886	4.8±3.0
Gynogens 40/45	694.6±57.5	2872	2.2±7.1

Note: * estimated from regression equations.

Unexpected low hatching rates were achieved from the breeding experiment in both groups which might have been because of low sperm quality. Normal hatching rates of Thai walking catfish range between 65-85%. However, when the induced-breeding experiment was repeated 2 months later using 45 females from each of control and gynogens groups, better results were obtained. Fourty-three gynogens and 44 control females responded to hormone injection and average hatching rates of eggs obtained from the gynogen and control groups were 41.8±16.2 and 43.5±11.7% respectively.

DISCUSSION

Suitable temperature for heat shock

The low incidence of diploid larvae with satisfactory hatching rates revealed that temperatures of 35 and 37°C were not high enough to induce retention of the second polar body of Thai walking catfish eggs. However, it is also possible that shock durations of 1-7 minutes were not long enough to complete the diploidization mechanism. Previous studies confirmed that low temperature with long shock duration might be as effective as high temperature with short durations in inducing polyploidy. However, different hatching success might result (Chourrout, 1980; Thorgaard and Hazwin, 1981 and Arai and Wilkins, 1987). Heat shocks applied for 3 minutes at 40°C and 1 minute at 42°C yielded similar rates of diploid fry. The diploid yield of the 42°C-group was significantly higher than that of the 40°C-group due to a high hatching percentage. This might be because high temperature applied for a short time was not so harmful.

Comparison of heat-shock and cold-shock

Comparison between cold shock and heat shock showed that heat shock was more harmful and less effective than cold shock in inducing diploidization of walking catfish gynogens.

The reason behind this is unknown. Perhaps the difference between shock and ambient temperature is a key factor determining the sucuss of polyploid induction. Therefore, the most effective temperature could not be identified for heat shock since it exceeded a vital temperature for egg development. Meanwhile, shock temperature could be lowered to a great extent so that the most effective temperature for cold shock was achieved with a minor effect on hatching success. Although no comparative study has been conducted in other silurids, a similar trend was shown in channel catfish in which a hatching success of 79% triploids was obtained when eggs were subjected to cold shock at 5°C for 60 minutes (Wolters *et al.*, 1981) while heat shock at 41°C applied for 3 minutes yielded only 62% triploidy with a hatching percentage of 38-42% (Bidwell *et al.*, 1985).

Hussian *et al.* (1991) had, however, suggested that heat shock should be applied earlier than cold shock since it accelerated the rate of all biological process. The timing of heat shock applied was in the optimum range according to preliminary research which indicated that the optimum initiation time for heat shock and cold shock of Thai walking catfish eggs was the same (Na-Nakorn, unpublished data).

The variability of the results obtained from heat shock and cold shock was similar. Teskeredzic *et al.* (1993) proposed that the inconsistency might be due to different sizes of eggs. Eggs of smaller diameter might receive greater temperature shocks than larger diameter eggs within the same treatment batch. Moreover, time to start heat shock is critical (Hussion *et al.*, 1991), therefore the same initiation time with different ambient temperatures prior to heat shock probably gave rise to different results.

The results reported here were contradictory to those reported for Nile tilapia by Hussian *et al.* (1991) where more consistent and better triploid yields were achieved from heat shock rather than cold shock. This probably indicated a species-specific response.

The yield of diploid gynogens obtained from cold shock in this study was lower than that reported earlier (Na-Nakorn *et al.*, 1993*b*). This may be due to different ambient temperature prior to cold shock or different tendencies to retain the second polar body in female Thai walking catfish (Na-Nakorn *et al.*, 1993*b*).

Growth and survival rate of the gynogens

Growth of gynogens was found to be inferior to the respective control in many species of fish such as plaice, *Pleuronectes platessa* (Purdom, 1969), coho salmon, *Oncorhynchus kisutch* (Refstie *et al.*, 1982), cyprinid loach, *Misgurnus anguillicaudatus* (Suzuki *et al.*, 1985) and common carp (Komen *et al.*, 1992) and inbreeding depression was considered to account for the results. In the present study, although differences between the gynogens and control were not distinct, inbreeding depression may exist with less severe effect due to species difference. Differences in culturing conditions may also explain the results. Communal stocking of gynogens and controls as practised by Suzuki *et al.* (1985) might lead to a certain level of competition which explains the effects of inbreeding depression. It has also been mentioned that highly homozygous individuals were more sensitive to varying environment (Falconer, 1981).

Size variation was greater in the gynogenetic Thai walking catfish than in the controls. Increase in variation of a particular trait characterizes inbreeding which results in a loss of heterozygous genotypes and increased frequency of homozygous genotypes representing more extreme phenotypic values (Falconer, 1981). Similar results were reported in common carp where a considerable increase in phenotypic variation was noted for body weight and gonad weight of meiotic and mitotic gynogens (Komen *et al.*, 1993).

Six-month-old female fish had swollen bellies due to ovarian development although they were not fully mature.

Sizes of males in the control group were slightly smaller than the control-females but the difference was not significant ($p>0.05$). The slightly greater weight of females might be due to the presence of eggs. Recently, a similar result was obtained when a comparison was made in different stock of 1-year-old Thai walking catfish (Na-Nakorn, unpublished data).

Survival rate of the mixed-sex control group did not differ greatly from that of the gynogens although among the latter it was slightly lower. A number of studies have shown that survival of gynogens is always reduced due to inbreeding depression (Purdom, 1969; Chourrout and Quillet, 1982 and Suzuki *et al.*, 1985). It might occur in the gynogenetic walking catfish during the early stage of fry which was not investigated.

Sexual types of the gynogens

All female gynogens (219 fish) obtained in this study suggest a female homogametic type of sex-determination in Thai walking catfish. Similar conclusions have been drawn in species such as common carp, grass carp and coho salmon (Nagy *et al.*, 1978; Stanley, 1976 and Refstie *et al.*, 1982). However, unexpected males have been found in some experiments such as in cyprinid loach (Suzuki *et al.*, 1985) and common carp (Komen *et al.*, 1992). Komen *et al.* (1992) proposed a homozygosity for a recessive mutation, termed *mas*-1 gene to explain their results in common carp in which a female homozygous for *mas*–+ gene or heterozygous *mas*–+/*mas*–1 differentiated into female while XX individual with homozygous *mas*-1 gene differentiated into male. Moreover, Oshiro (1987) reported a different sex ratio of gynogenetic goldfish (*Carassius auratus*) obtained from different females although it was shown to be a female homogametic species. Changes of genetic sex determination system due to long-term selection were proposed to account for the result.

Effects of inbreeding on gonadal development and ovulation response

Effects of gynogenetic inbreeding on gonad development of Thai walking catfish were not extreme. Inbreeding slightly reduced GSI value, probably through a marked reduction of fecundity. Oogenesis seemed not to be seriously affected since all the ovaries investigated were dominated with maturing oocytes characterized by a dark-brown colouration instead of a yellowish one. Slight reduction of the ovulation response after hormone injection was observed in the gynogens. This finding was in agreement with that of Komen *et al.* (1992) who observed a close

relationship between a reduction of ovulation response and increasing levels of inbreeding in common carp.

Potential use of the gynogenetic Thai walking catfish for aquaculture

The idea of using all-female gynogenetic stock of Thai walking catfish to directly enhance production of this species is doubtful. However, potentiel use for breeding purposes is still of interest providing that the effects of inbreeding– reduction of fecundity and fertility– and so on are eliminated. This can be done by production of functional XX males, which can be achieved through androgen treatment and crossing of the sex-reversed gynogenetic males with gynogenetic-females of different strain (Nagy *et al.*, 1984 and Komen *et al.*, 1993).

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