
**ISSN: 2347-5129**
IJFAS 2014; 1(6): 151-162
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www.fisheriesjournal.com
Received: 27-05-2014
Accepted: 10-06-2014

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# Triploidy induction and growth performance of hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*)

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**Abstract**
Cold shock and heat shock were evaluated for their efficacy as methods of manipulating ploidy in hybrid catfish (*C. macrocephalus* × *C. gariepinus*). The most effective method of inducing triploidy was cold shock at 6 °C applied of 25 min which resulted in 87% triploidy with 48% fry survival rate from fertilization to two weeks rearing period. Both of thermal shock treatment initiated 3 minutes after water activation. Cold shock treatment for 30 min or more caused total mortality of embryos. Heat shock at 40 °C for 1 min resulted 42% triploidy with fry survival of 42%. Flow cytometry analysis was used to assess the ploidy level of diploid and triploid larvae in both experiments with blood samples. In growth experiment, growth of triploid was higher than control but lower than the cold shocked (6 °C 25 min) diploid fry, with the survival rate ranging between 63-73% in all treatments after 16 weeks of rearing period. Triploid fish were 15% larger than control; while shocked diploid was 27% larger than control.

**Keywords:** Cold shock, heat shock, triploidy, embryo, mortality, Flow cytometry, triploid, shocked diploid, control.

## 1. Introduction
Hybrid *Clarias* catfish (*Clarias macrocephalus* × *Clarias gariepinus*) is a cultured fish of major economic importance in Thailand. Not long past, the hybrid catfish has faced slow growth and poor survival rate. Hybridization is a dormant major problem in seed production and hatchery management. The performance of hybrid catfish has become uncertain, resulting in poorer returns for grow-out farms. It is believed that this uncertain performance is linked to the poor quality of the hybrid *Clarias* catfish fry. To compensate the loss, farmers are using higher stocking rates to compensate for mortality of fry. Higher stocking rate of fry may also cause slower growth rates, increasing the feed conversion ratio and the time to harvest marketable fish. Ingthamjitr (1997) [1] that these factors increase both the risk and costs to the farmers, reducing both predictability and returns.
Generally, the hybrid of interspecific hybridization is sterile. The hybrid catfish (*C. macrocephalus x C. gariepinus*) females are fertile and potentially capable of making large numbers of backcross progeny. However, Abol-Munafi *et al.* (2004) [2] expressed hybrid male is unsuccessful in producing F2 and backcross hybrid, which could probably be due to the problem of spermatogenesis. If female escape from any culture system, then it can reproduce and destroy the wild stock. These problems may be solved by triploidy induction, which produces sterile and faster growing individuals.
As a technique of chromosome engineering, triploidy induction has a special interest in producing sterile populations of fish. Sexual development of fish reduces somatic growth. Purdom (1972) [3] revealed that a major part of the nutrient and energy is used for sexual maturation. Sterilization may overcome the detrimental effects of sexual maturation on normal growth of fish. Hunter and Donaldson (1983) [4] expressed that sterility in fish can be induced either by exogenous hormone treatment or by triploidy induction (Thorgaard, 1983; Hussain *et al*., 1981; Hussain, 1996) [5, 6, 7].
The main reasons for inducing triploidy usually related to either sterility (triploids are functionally sterile), improved growth performance, or some other improved triploid quality. Triploids are sterile and can be released to the wild without danger of becoming established (exotic introductions) or of contaminating local gene pools where the species in question is established. This is an important consideration where cultured species have been altered through

genetic selection. Often, but not always, triploids grow faster compared with diploids. There is also evidence that with some species, triploids are less aggressive. Fast *et al*. (1998) [8] revealed triploidy induction combined with sex reversal is also a useful technique for developing all male or all female captives.

In Thailand the culture period of hybrid catfish in grow-out pond is 6 months (Testhong Farm, Personal communication). If the fish become tripled then maybe it needs less time to become a marketable size, which was the study area of this thesis. If the culture period can be reduced then feeding cost and other operational costs will be reduced and it will make the hatchery more profitable. To overcome the problem, to produce genetically sterile hybrid stock has been identified as a potential approach towards shortening the culture period. In this study I attempt to induce triploidy in Clarias catfish using both cold and heat shock. I also attempt to know the optimum combination of temperature and duration in triploidy induction along with the hatching, survival of the triploid and shocked dipoloid catfish.

## 2. Materials and Methods

### 2.1 Brood fish collection, maintenance and handling

The experiments were conducted in National aquaculture Genetic Research Institute (NAGRI), AARM hatchery and the Aquaculture research facility at the Asian Institute of Technology, Thailand. For this experiment the brood fish was collected from Testhong farm. The size of the female brood stock was 200-250 g size/fish and the age of about 1 year. The male brood stock was 3-5 kg size/fish and the age of about 2-3 year.

### 2.2 Induced breeding/Artificial fertilization

To induce ovulation in the female, hormone of the Chinese carp's pituitary gland was used. There was no necessity to induce the male for milt production. The needed amount of pituitary and doses were calculated. For female; first injection was given as ½ of pituitary gland per kg fish; second injection was decided as one pituitary gland per kg fish. One kilogram of fish was injected with 1 ml of solvent requirement. The fish was injected with the appropriate dose intramuscularly (dorsal fin). The hypophysation of the female was done in two stages; first injection was given 14 hours before the removal of eggs; second injection was given 8 hours before the removal of eggs.

### 2.3 Hybridization

After finishing the second injection the female brood fish were carried out to AIT for the hybridization and following hybridization procedures were followed:

Females were stripped after 8 hours of the second injection and placed into a bowl. Males usually cannot strip because the male testis have so many grooves for the sperm to come out. The male was sacrificed to collect sperm from the testis. So, male was used here as a donor fish Milt spilt over stripped eggs and stirred with feather continuously for 1 minute Dry method (eggs and sperm mixed together before the water was added) were followed in fertilization. Clean water was added and stirred for a few minutes and drained water. This will be repeated for 3 times.

### 2.4 Triploidy induction by cold and heat shock

Immediately after fertilization and before they began to adhere, the fertilized eggs were distributed into the strainer by using micropipette.1 micro-liter of eggs were distributed in 3 Petri dishes and counted to take an idea about the total amount of eggs present in each strainer. The average was used to estimate the amount of eggs in each Petri dish. A minimum of 320 eggs was subjected to cold shock at 2 $^{o}$C, 4 $^{o}$C and 6 $^{o}$C that started 3 min after water activation of the eggs and lasted for 10, 20 and 25 minutes shock duration. Consequently, the similar procedures were followed for the heat shock. The eggs from other females were heat shocked at 38 $^{o}$C, 40 $^{o}$C and 42 $^{o}$C starting at 3 min after water activation with the shock duration of 1 min, 2.5 and 4 min. Another treatment at room temperature was served as the control for both cold and heat shock temperature. There were 3 replicates of each treatment. Then the strainers containing the eggs were placed in thermostatically controlled water bath after treated the eggs were re-warmed for 5 minutes using water at room temperature and then transferred to the hatching hapa with aerated well water and flow through systems. Time of hatching was dependent on water temperature. The normal water temperature was about 30.2 $^{o}$C, the time from fertilization until hatching was ~ 24 hour.

Fertilization rate was calculated based on the number of white eggs, which were considered not fertilized. Thirty (30) hours after insemination, hatchlings were counted and the hatching rate was calculated as the relative percentages of the initial eggs were fertilized and incubated. After 24-30 hrs, the percentages of normal and deformed larvae and of non-developed eggs were calculated (Manickam and Joy, 1989) and found that there were no deformed larvae. Survival rate was assessed two (2) weeks after hatching.

### 2.5 Flow Cytometry Techniques to assess the triploidy:

The fry were reared for 15 days and brought them to the National Aquaculture Genetic Research Institute to assess the triploidy by using the Flow cytometry machine (PARTEC PA ll). The fry were 15 days old and sacrificed to collect the blood samples by using micro-hematocrit tubes. The micro-hematocrit tubes were suspended in the SARSTEDT tube containing 1 ml of PARTEC Cystein UV ploidy (DAPI staining solution). The sample was carried out to the Flow cytometry machine room to detect the triploidy. Triploid were determined by automated peak evaluation. When the fry was diploid it was shown the peak in 200 and if the fry was triploid it was shown that the peak is in 300, which means that 2n have less DNA content than 3n

### 2.6 Growth measurement

The optimum rate of temperature and duration for triploidy induction was determined by considering two factors: The highest rate of triploidy induction and the maximum survival rate from hatching to 15 days rearing period. 6 $^{o}$C 25 min and 40 $^{o}$C gave the highest triploidy rate. So, these treatments were used for the growth experiment. 100 females and 6 males were used to induce triploidy at 6 and 40 $^{o}$C. A large number of eggs were used for the shock induction. After hatching, hatchlings were reared for 35 days to let them grow enough to collect the blood sample. Sufficient amount of blood was required to determine the triploidy and cold and heat shocked diploid fry. At 6 $^{o}$C 25 min sufficient amount of triploid and shocked diploid fry were found. At 40 $^{o}$C 1 min no triploidy fry were found. There were three replicates in each treatment. So, there were twelve tanks in this experiment. The fry were reared in large concrete tank with water recirculation systems

and the fry were feeding with live and frozen *Moina* sp. 8 times/day.

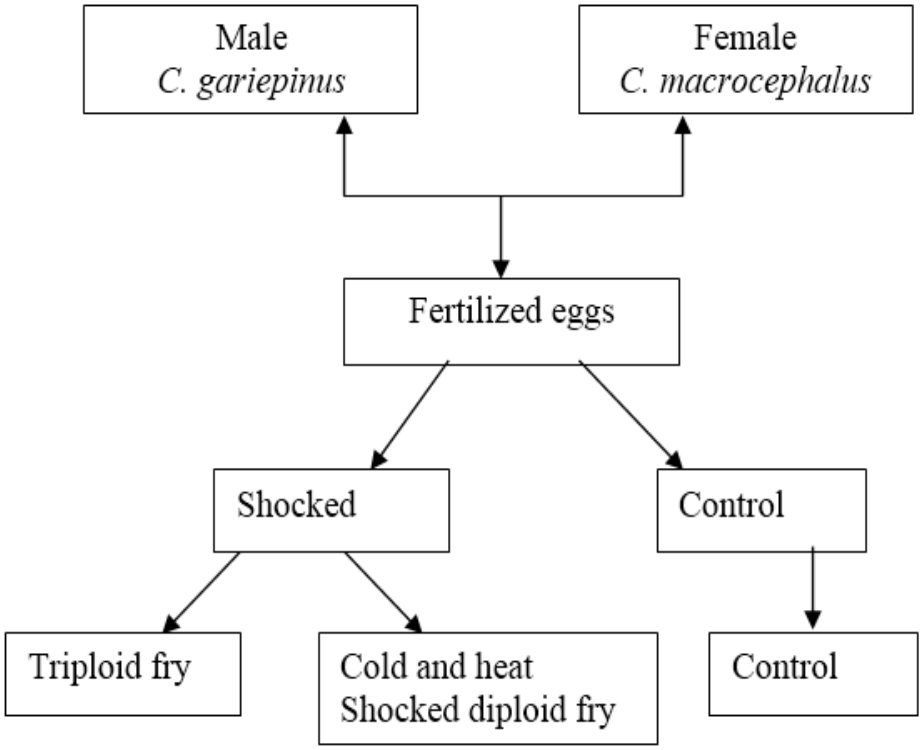


**Fig 1:** Experimental framework for the comparison of growth and survival of triploid, shocked diploid and normal diploid

After rearing more than 1 month the fry were carried out to NAGRI to determine the triploidy and shocked diploid fry. Triploidy and cold shocked diploid were separated by using fish one by one. After separating, the fry were brought back to AIT for further growth observation. 136 fry were distributed in each tank and rearing them for 75 days. Feeding frequency was 4-5 times/ day. The fry were feed frog feed containing 40% protein at a feeding rate of 20% BW. The total amount of food was given slowly to the tank and observed until they satiated. For the growth experiment the following parameters were observed:

These parameters were used to evaluate the growth performance.

A. Daily weight gain (g fish/day)

$$= \frac{\text{Mean final weight (g)} - \text{Mean initial weight (g)}}{\text{Period (days)}}$$

B. Specific growth rate (SGR, % day-1)= 100* (ln Wf – ln Wi) /t

Where, Wf is final weight, Wi is initial weight, t is time (No. of days for the experiment)

C) Survival rate = $\frac{\text{Final no. of fish}}{\text{Initial no. of fish}} * 100$

Experimental fish were weighed before releasing into the tank at the beginning and thirty fish sampling was taken by weekly. At the end of the experiment, all fish in each tank were weighed and counted for the survival rate

### 2.7 Statistical analysis

The results of the various trials will be statistically compared using a test of homogeneity of variances before ANOVA followed by Turkey's Multiple Range test. Mean will be given with ± standard error (SE).

## 3. Results

### 3.1 Triploidy induction using Cold Shock and Heat shock

In this experiment, fertilization rate was 100% for cold and heat shock. Hatching rate was highest (82%) in the control (30.2 $^{o}$C) followed by cold shock at 4 and 6 $^{o}$C for 10 minutes (48 and 54%, respectively) but significantly lower at 2 $^{o}$C (Fig. 1.1)

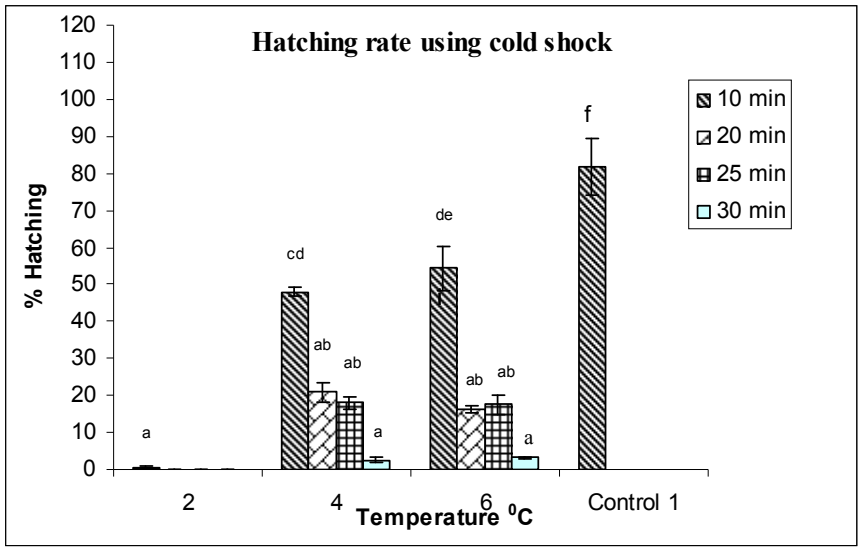


**Fig 1.1:** Effect of cold shock on hatching of hybrid catfish

In the case of heat shock, the control (30.2 $^{o}$C) also gave the highest hatching rate followed by 38 $^{o}$C groups (1, 2.5 and 4 min) and 40 $^{o}$C (1 min) treatment. Heat shock duration of 2.5 and 4 min at 40 and 42 $^{o}$C caused 100% or nearly 100% mortality of eggs (Fig. 1.2).

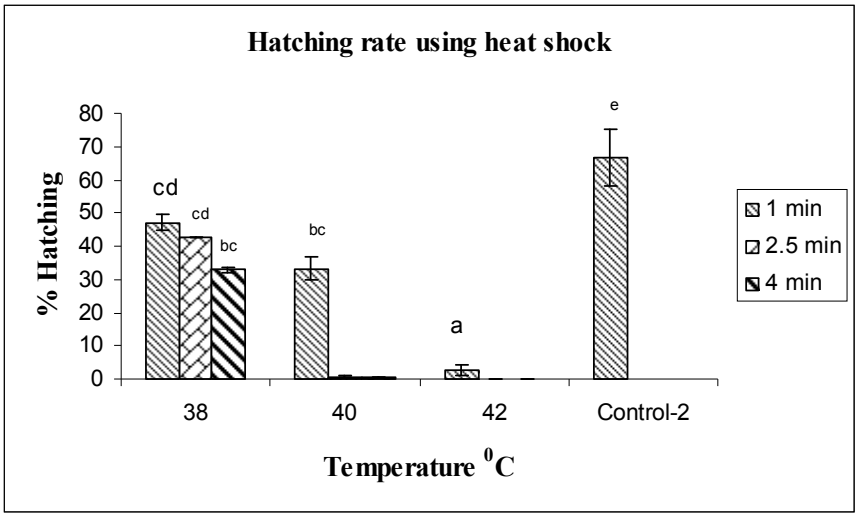


**Fig 1.2:** Effect of heat shock on hatching of hybrid catfish

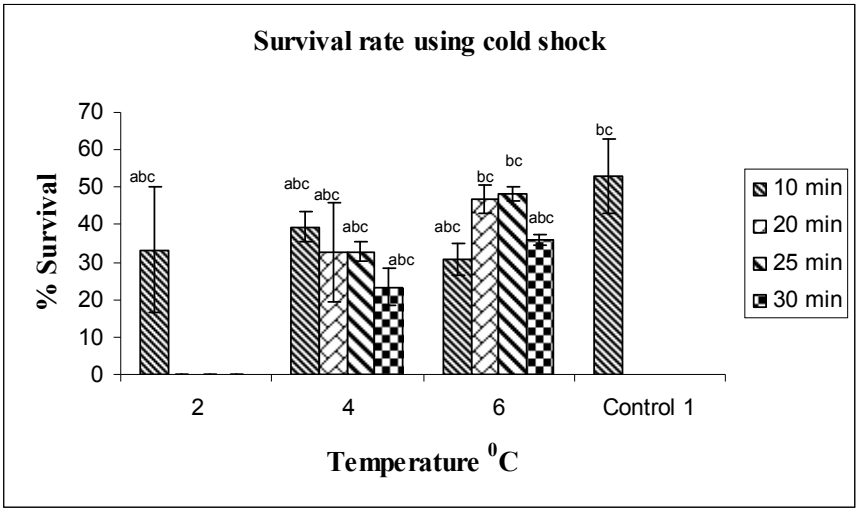


**Fig 1.3:** Effect of cold shock on survival of hybrid catfish

Figure 1.3 show that hatchlings produced using cold shock did not have significant difference in survival rates between treatments. In heat shock treatments, survival rates of hatchlings that were shocked at 38 and 40 $^{o}$C were as high as that of the control (30.2 $^{o}$C) but survival rate of hatchlings produced at 42 $^{o}$C were significantly lower than that of the control (Fig. 1.4).

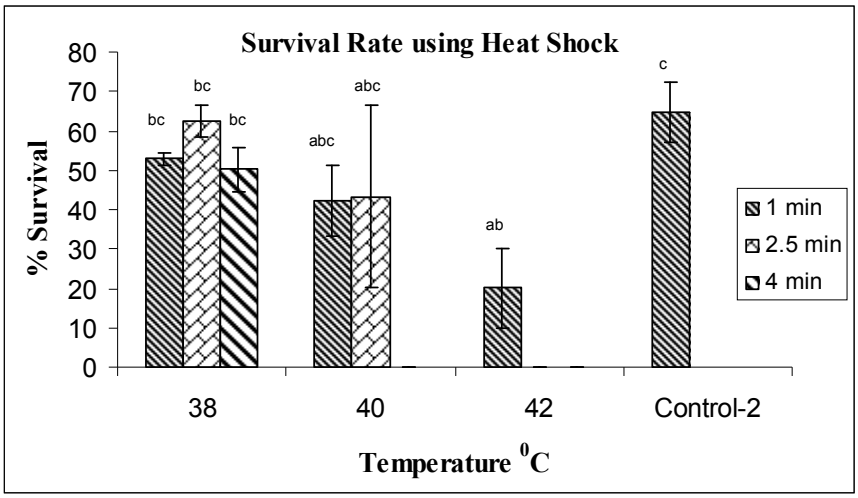


**Fig 1.4:** Effect of heat shock on survival of hybrid catfish

Ploidy level was assessed by flow cytometry analysis which determine rapidly and accurately the ploidy status in hybrid catfish larval. Triploidy rate was highest (87%) at 6 $^{o}$C 25 minutes followed by 4 $^{o}$C 25 min (Fig. 1.5). In heat shock treatment, only 40 $^{o}$C 1 min treatment resulted in triploidy induction 42%.

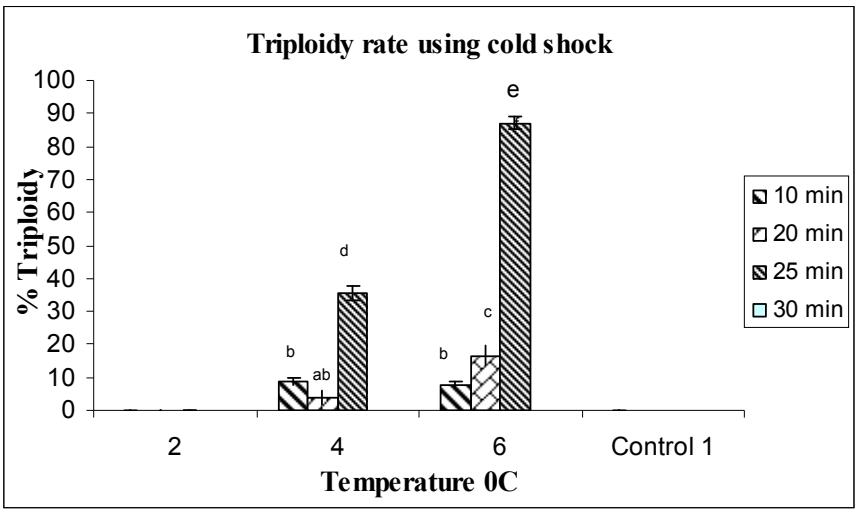


**Fig 1.5:** Effect of cold shock on triploidy of hybrid catfish fry

**Suitable temperature and shock duration for triploidy induction using heat and cold shock**

Table 1 shows the summary of results. Cold shock at 2 $^{o}$C (10, 20, 25, 30 min) had no hatching success at all. In case of 4$^{o}$ and 6 $^{o}$C (10 min) the hatching success was significantly higher than other treatments. Using these two at 20 and 25 min shock induction, the hatching rate was considerably lower than at 10 min. The shock temperatures from 2-6 $^{o}$C at 30 min shock duration resulted no hatching of fertilized eggs. Although the hatching rate was not high at 6 $^{o}$C 25 min, the triploidy rate was significantly higher at this temperature and duration followed by 4 $^{o}$C 25 min. So, to get the better triploidy rate it can recommend 6 $^{o}$C 25 min as the best cold shock treatment.

**Table 1:** Hatching, survival and triploidy rates of hybrid catfish induced using cold and heat shock. The results are as present as mean and standard error (mean ± SE)

| S. No. | Treatment | Hatching | Survival | Triploidy |
|---|---|---|---|---|
| 1 | 2 °C 10 min | 0.63 ± 0.36$^{a}$ | 33.3 ± 16.7$^{abc}$ | 0$^{a}$ |
| 2 | 2 °C 20 min | 0.10 ± 0.10$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| 3 | 2 °C 25 min | 0.10 ± 0.10$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| 4 | 2 °C 30 min | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| 5 | 4 °C 10 min | 48.02 ± 1.37$^{cd}$ | 39.48 ± 3.89$^{abc}$ | 8.89 ± 1.11$^{b}$ |
| 6 | 4 °C 20 min | 20.83 ± 2.80$^{ab}$ | 32.51 ± 13.30$^{abc}$ | 3.81 ±1.98$^{ab}$ |
| 7 | 4 °C 25 min | 18.02 ± 1.62$^{ab}$ | 32.81 ± 2.55$^{abc}$ | 35.77 ± 2.18$^{d}$ |
| 8 | 4 °C 30min | 2.56 ± .85$^{a}$ | 23.33 ± 5.09$^{abc}$ | 0$^{a}$ |
| 9 | 6 °C 10 min | 54.27 ± 6.05$^{de}$ | 30.81 ± 4.26$^{abc}$ | 7.71 ± 1.11$^{b}$ |
| 10 | 6 °C 20 min | 16.35 ± .91$^{ab}$ | 46.90 ± 3.71$^{bc}$ | 16.58 ± 2.83$^{c}$ |
| 11 | 6 °C 25 min | 17.50 ± 2.66$^{ab}$ | 48.46 ± 1.90$^{bc}$ | 87.10 ± 2.07$^{e}$ |
| 12 | 6 °C 30 min | 3.13 ± .33$^{a}$ | 36.05 ± 1.49$^{abc}$ | 0$^{a}$ |
| 13 | 30.2 °C(Contl-1) | 81.88 ± 7.55$^{f}$ | 53.03 ± 9.96$^{bc}$ | 0$^{a}$ |
| 14 | 38 °C 1.0 min | 47.16± 2.49$^{cd}$ | 52.82 ± 1.47$^{bc}$ | 0$^{a}$ |
| 15 | 38 °C 2.5 min | 42.59 ± .1$^{cd}$ | 62.40 ± 4.02$^{bc}$ | 0$^{a}$ |
| 16 | 38 °C 4.0 min | 32.94 ± .81$^{bc}$ | 50.12 ± 5.51$^{bc}$ | 0$^{a}$ |
| 17 | 40 °C 1.0 min | 33.33 ± 3.45$^{bc}$ | 42.27 ± 9.07$^{abc}$ | 41.98±2.35$^{d}$ |
| 18 | 40 °C 2.5 min | .80 ± .36$^{a}$ | 43.33 ± 23.33$^{abc}$ | 0$^{a}$ |
| 19 | 40 °C 4.0 min | .60 ± .17$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| 20 | 42 °C 1.0 min | 2.59 ± 1.66$^{a}$ | 20.05 ± 10.06$^{ab}$ | 0$^{a}$ |
| 21 | 42 °C 2.5 min | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| 22 | 42 °C 4.0 min | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ |
| 23 | 30.2 °C (Contl-2) | 66.67 ± 8.45$^{e}$ | 64.61 ± 7.75$^{c}$ | 0$^{a}$ |

In case of heat shock it was found that 38 °C (1- 4 min) resulted in the highest hatching percentage followed by 40 °C (1, 2.5 min). Heat shock duration of 4 min at 40 and 42 °C caused 100 % mortality of the fertilized eggs. In this experiment 40 °C 1 min was the best treatment for hatching and triploidy.

Figure 1.6 represents the results on the application of cold and heat shock at six temperature regimes to induce triploidy. Table 4.1 indicates that of the six temperatures tested 6 °C gave the highest percentage of triploidy (range 87%) when the shock was applied three minutes after fertilization for 25 min. From 25 min onward there was a very high mortality of embryos. In contrast, the triploidy rate at 40 °C was considerably lower; the highest level achieved being only amount 42% as against 87% at 6 °C.

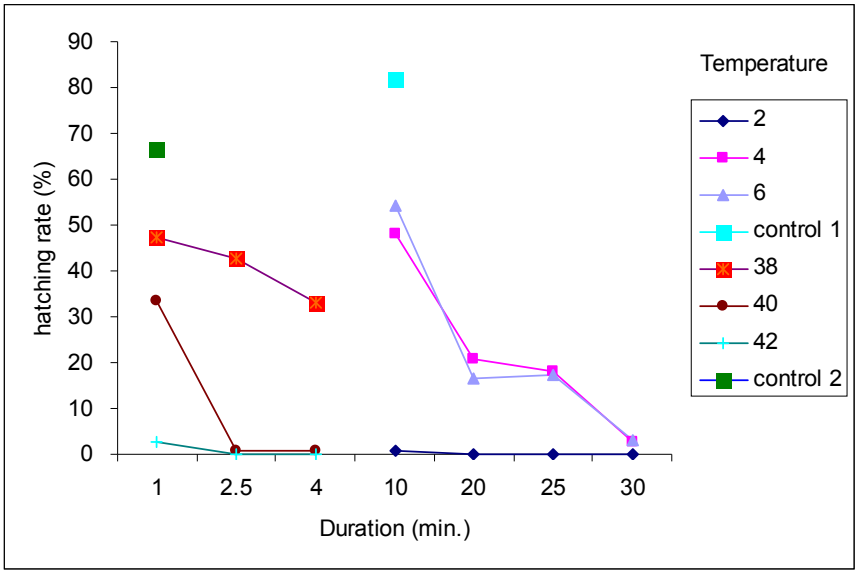


**Fig 1.6:** Interaction in hatching between temperature and duration

Although longer shock durations at 4 ºC and 6 ºC increased the rate of triploidy induction, they generally had an adverse effect on hatching and survival performance. Figure 4.6 shows that there was no hatching rate at 2 ºC. Statistical analysis reveals that the hatching performance at 10 minutes of 4 and 6 ºC were not significantly different. Table 4.1 shows that hatchlings produced at cold shock of 25 and 20 min at 6 ºC had the highest survival rate and in case of heat shock of 2.5 min at 38 ºC had the highest survival rate. Both below and above this duration the survival was lower. Statistically, the cold shock duration of 20 and 25 min at 6 ºC and 4 ºC was not significantly different. Thus considering all the survival and triploidy rates at 6 ºC, 20 and 20 min cold shock treatment was found to be the best; whereas at 4 ºC 20 and 25 min were the most effective.

Hatching and survival rates of cold shock treated groups were lower than those of control groups. Statistical analysis showed that there were interactions between treatments.

### 3.2 Growth performance of hybrid catfish

During the whole experimental period (75 days) cold shocked (6 ºC 25 min) diploid fry had given the highest body weight.

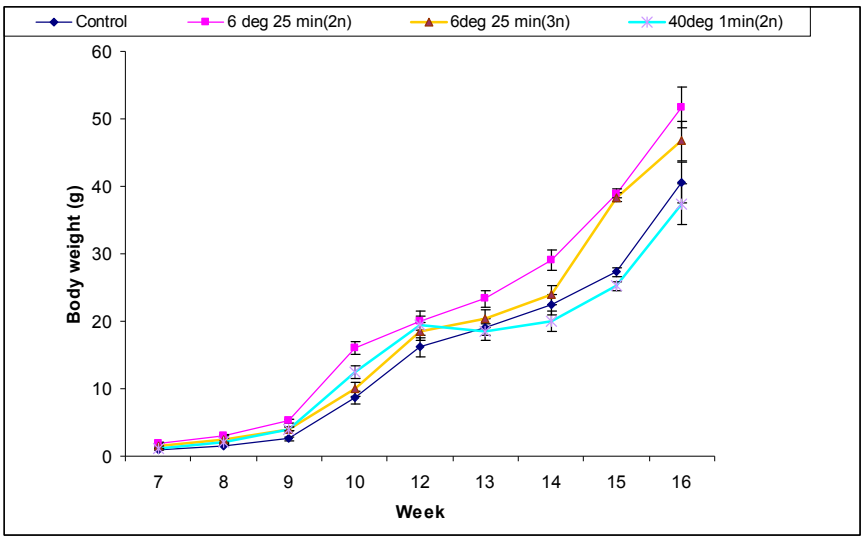


**Fig 1.7:** Mean body weight of hybrid catfish fry (after determining the triploid and shocked diploid fry by flow cytometry) during the whole experimental period, 75 days.

Triploid and shocked diploid (6 ºC 25 min) fry had significantly higher body weight and body length than the control. Cold shocked diploid (6 ºC 25 min) fry had continuously significant highest body weight than all other treatments from seven to ten week. Triploidy, cold and heat shocked diploid (6 ºC 25 min and 40 ºC 1 min) fry had significantly higher body weight than control (Fig. 1.7).

Cold shocked diploid (6 ºC 25 min) fry had retained the highest body weight again after thirteen week, which was significantly different than other treatments. Cold shocked diploid (6 ºC 25 min) fry had significantly higher body weight than all other treatments after fourteen week and in this week control and heat shocked diploid (40 ºC 1 min) fry had significantly same body weight. Triploidy and cold shocked (6 ºC 25 min) diploid had the same body weight in 15 week rearing period which was significantly higher than control and heat shocked (40 ºC 1 min) diploid fry (Fig 1.7)

Body weight of cold shocked diploid (6 ºC 25 min) fry had significantly higher body weight than all other treatments. Triploid had also the significant higher body weight than control after 16 weeks. In case of control and heat shocked (40 ºC 1 min) diploid fry there were no significant difference (Fig. 1.7).

Triploid and cold shocked diploid (6 ºC 25 min) fry gave significantly higher body length than the control and another heat shocked diploid fry (40 ºC 1 min) after seven and eight week (Fig 1.8). Cold shocked (6 ºC 25 min) diploid fry had the most significant higher body length after nine weeks. After ten and twelve weeks of rearing cold shocked (6 ºC 25 min) diploid fry had the highest body length than control, triploid and heat shocked (40 $^{0}$C 1 min) diploid fry. There were no significant length difference between control, triploidy and heat shocked diploid (40 ºC 1 min) fry after thirteen week. Body length was significantly higher than other treatments in cold shocked (6 ºC 25 min) diploid fry after 14 week. Cold shocked diploid (6 ºC 25 min) and triploid fry had the significant body length after 15 weeks of rearing. Triploidy and cold shocked (6 ºC 25 min) diploid fry had

similar body length which was significantly higher than control and heat shocked (40 ºC 1 min) diploid fry. Table 2 shows the summary of results. Triploidy gave the highest body weight than control but cold shocked diploid gave the best growth performance than triploids during the whole experimental period.

**Table 2:** Comparison of Body weight (gm) and Body length (cm) in Triploidy (3n), shocked induced diploids (2n) and control in different weeks .The results are as presented as mean and standard error (mean ± SE)

| No | Treatment | Week | Bodyweight (g) | Body Length (cm) |
|---|---|---|---|---|
| 1 | Control | 7th | 0.94 ± .05a | 4.67 ± .09a |
| 2 | 6 0C 25 min (2n) | 7th | 1.92 ± .048d | 6.21 ± .07c |
| 3 | 6 0C 25 min (3n) | 7th | 1.48 ± .06c | 5.43 ± .08b |
| 4 | 40 0C 1 min (2n) | 7th | 1.14 ± .04b | 4.95 ± .08a |
| 5 | Control | 8th | 1.58 ± .04a | 5.86 ± .08a |
| 6 | 6 0C 25 min (2n) | 8th | 3.08 ± .06d | 7.46 ± .06b |
| 7 | 6 0C 25 min (3n) | 8th | 2.46 ± .09c | 7.52 ± .71b |
| 8 | 40 0C 1 min (2n) | 8th | 2.00 ± .07b | 6.28 ± .08ab |
| 5 | Control | 9th | 1.58 ± .04a | 5.86 ± .08a |
| 6 | 6 0C 25 min (2n) | 9th | 3.08 ± .06d | 7.46 ± .06b |
| 7 | 6 0C 25 min (3n) | 9th | 2.46 ± .09c | 7.52 ± .71b |
| 8 | 40 0C 1 min (2n) | 9th | 2.00 ± .07b | 6.28 ± .08ab |
| 9 | Control | 10th | 2.64 ± .10a | 6.86 ± .1a |
| 10 | 6 0C 25 min (2n) | 10th | 5.19 ± .12c | 8.50 ± .07c |
| 11 | 6 0C 25 min (3n) | 10th | 4.04 ± .14b | 7.95 ± .1b |
| 12 | 40 0C 1 min (2n) | 10th | 3.99 ± .12b | 7.67 ± .1b |
| 13 | Control | 12th | 8.56 ± .25a | 10.15 ± .11a |
| 14 | 6 0C 25 min (2n) | 12th | 16.07 ± .25d | 12.05 ± .1d |
| 15 | 60C 25 min (3n) | 12th | 9.92 ± .19b | 10.61 ± .1b |
| 16 | 40 0C 1 min (2n) | 12th | 12.59 ± .34c | 11.15 ± .12c |
| 17 | Control | 13th | 16.13 ± .63a | 12.10 ± .16a |
| 18 | 6 0C 25 min (2n) | 13th | 20.05 ± .62b | 13.03 ± .15c |
| 19 | 6 0C 25 min (3n) | 13th | 18.47 ± .62b | 12.42 ± .14ab |
| 20 | 40 0C 1 min (2n) | 13th | 19.37 ± .62b | 12.92 ± .18bc |
| 21 | Control | 14th | 19.13 ± .65a | 12.83 ± .16a |
| 22 | 6 0C 25 min (2n) | 14th | 23.35 ± .74b | 13.94 ± .19b |
| 23 | 6 0C 25 min (3n) | 14th | 20.40 ± .77a | 13.15 ± .2a |
| 24 | 40 0C 1 min (2n) | 14th | 18.45 ± .82a | 12.61 ± .2a |
| 25 | Control | 15th | 22.41 ± .75ab | 14.29 ± .21c |
| 26 | 6 0C 25 min (2n) | 15th | 29.10 ± .88d | 15.15 ± .19d |
| 27 | 6 0C 25 min(3n) | 15th | 23.87 ± 1.02c | 14.00 ± .20bc |
| 28 | 40 0C 1 min (2n) | 15th | 19.99 ± .81a | 13.37 ± 2a |
| 29 | Control | 16th | 27.33 ± .77a | 15.13 ± .19a |
| 30 | 6 0C 25 min (2n) | 16th | 38.90 ± 1.19b | 17.02 ± .21c |
| 31 | 6 0C 25 min (3n) | 16th | 38.34 ± 1.33b | 16.24 ± .23b |
| 32 | 40 0C 1 min (2n) | 16th | 25.33± .83a | 14.52 ± .21a |

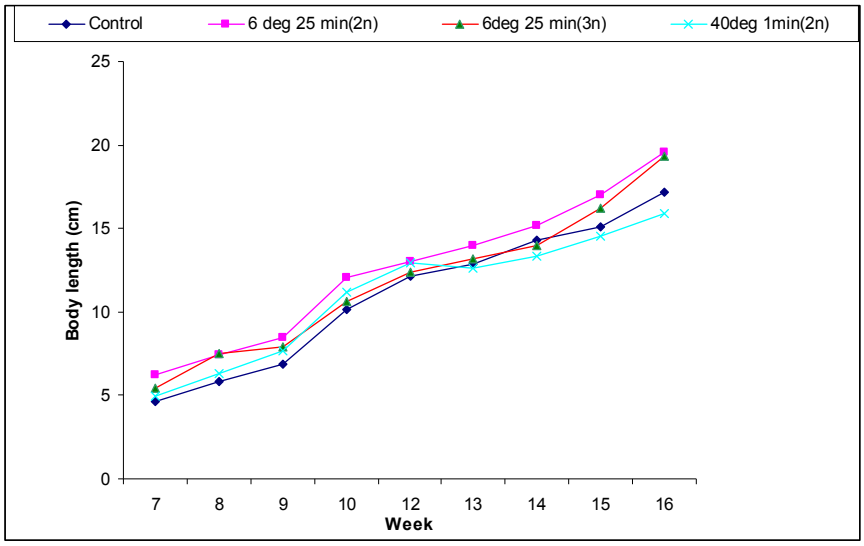


**Fig 1.8:** Mean body length of hybrid catfish fry (after determining the triploid and shocked diploid fry by flow cytometry) during the whole experimental period, 75 days.

Table 3 shows the significant difference between all other treatments for daily weight gain, specific growth rate and survival rate of hybrid catfish fry after ending the experiment.

**Table 3:** Daily weight gain (DWG), Specific growth rate (SGR) and Survival rate of different treatments (mean ± SE) [1]

| Treatment | Initial weight (g) | Final weight (g) | DWG ± SE (g day$^{-1}$) | SGR ± SE (% day $^{-1}$) | Survival rate (%) |
|---|---|---|---|---|---|
| Control | 0.3 ± .01$^{a}$ | 30 ± .43$^{a}$ | 0.39 ± .006$^{a}$ | 2.90 ± .05$^{a}$ | 62.99 ± 2.41$^{a}$ |
| 6 deg 25min (2n) | 0.6 ± .0$^{b}$ | 50 ± 2.15$^{b}$ | 0.71 ± .029$^{b}$ | 4.51 ± .07$^{b}$ | 59.56 ± .74$^{a}$ |
| 6 deg 25min (3n) | 0.34 ± .0$^{a}$ | 29 ± 3.39$^{a}$ | 0.38 ± .045$^{a}$ | 3.03 ± .18$^{a}$ | 70.1 ± 2.18$^{ab}$ |
| 40 deg 1 min (2n) | 0.33 ± .0$^{a}$ | 38 ± 1.59$^{a}$ | 0.51 ± .02$^{a}$ | 3.36 ± .08$^{a}$ | 72.79 ± 2.36$^{b}$ |

[1] Different letters in each column show significant difference at P< 0.05.

Growth was significantly higher in cold shocked (6 °C 25 min) diploid fry. During the experimental period fish increased with a mean 0.39, 0.71, 0.38, 0.51 g fish$^{-1}$ day$^{-1}$ for the treatment of control, triploidy, cold shocked (6 °C 25 min) diploids and heat shocked(40 °C 1 min) diploid (Table 4.3). Survival rate was higher in heat shocked (40 °C 1min) diploid fish which was followed by triploidy. Though the growth rate was higher in cold shocked (6 °C25 min) diploid fish, the survival rate was relatively lower. The survival rate of experimental fish ranged between 63-73% with significant difference among treatment groups

## 4. Discussion

### 4.1 Triploidy induction

The result of this experiment, suggests that cold shock may be the preferred method in hybrid catfish if triploidy production rate at or near 100% are required. Cold shock temperature of 6 °C 25 minutes resulted in 87% triploidy induction with hatching 17.5%. The highest triploidy induction rate that was observed using heat shock was 42% at 40 °C 1 min.

In case of 4 and 6 °C (10, 20, 25 and 30 min) the hatching success was 48.02- 2.56% and 54.27- 3.13% respectively. Cold shocks longer than 30 min caused total mortality in hybrid catfishes which was similar to Cassani and Caton (1985) [9]. In case of grass carp, *Ctenopharyngodon idella* cold shocks of 5-7 °C for 30 min or longer resulted in 100% mortality. Triploidy induction at cold temperature directly related to cold shock duration and inversely related to survival. Krasznai (1987) [10] found that cold shocks at 4 °C, longer than 1 h caused total mortality of European catfish, *Silirus glanis* L.

Manickam (1991) [11] found that triploidy induction in *C. batrachus* were subjected to cold shock at 5 °C for 1.0 h had an average hatching success of 94.2% and 91.2% respectively and yielded 100% triploidy as shown by chromosome counts. Hatching success of cold shocked eggs by 1.0 h was not significantly different from the control but longer durations of cold shock resulted in 100% mortality.

A general review of the different results reports that moderate temperature shocks at 6 °C (25 min) starting from 3 min after fertilization or more than 6 °C temperature would be the recommended treatment to

induce triploidy in hybrid catfish. These results are in agreement with recent studies of this species is by other scientists. The cold shock at 7 $^0$C starting 4.5 min after water activation was recommended by Na-Nakorn (1999) [12] induced 72%,74% and 88% triploid fish with 44-49% hatching success compared to the control (69-78%).

Temperature 38 $^o$C with satisfactory hatching rate revealed that this temperature is not high enough to induce the second polar body of hybrid catfish. Na- Nakorn (1995) [13] found that 35 and 37 $^o$C temperature also not enough to retention the second polar body of Thai walking catfish eggs but suggested that it might be possible that shock durations of 1-7 minutes were not long enough to complete the diplodization mechanism.

The previous study already confirmed that low temperature with long shock duration might be as effective as high temperature with short durations in inducing polyploidy which is as similar as the present study. The hatching success might be resulted different (Chourrout, 1980; Thorgaard 1986, Arai, 2000) [14, 15, 16].

Heat shocks applied for 1 min at 40 $^o$C yielded 41.98% triploidy but Chourrout and Quillet (1982) [17] obtained 100% triploids with a 26 $^o$C heat shock applied for 20 min, 25 min after fertilization, with survival rate to swim up no different from the controls.

The requirement for the induction of triploidy using heat shock is the optimal age of zygote, temperature level and duration of thermal shock which is investigated in a series of experiment. A maximum of 41% triploids were obtained when the fertilized eggs (3-min old) were heat shocked at 40 $^o$C 1- min duration.

Heat shock more than 4 min causes the 100 % mortality of hybrid catfish eggs but Haniffa *et al*., (2004) [18] reported that in case of stinging catfish, *Heterropneustes fossilis* (Bloch) a maximum of 82 ± 7% triploids were obtained at 40 $^o$C 4 min when the eggs were 2.5 min old after fertilization. It was also mentioned that heat shock above 42 $^o$C at all duration resulted in 100% mortality of stinging catfish embryos. In this experiment heat shock at 40 $^o$C 2.5 min or above that caused the total mortality.

Before determining the triploidy rate by flow cytometry, the survival rate of two weeks cold (4 and 6 $^o$C) and heat shocked fry give the lower survival than control, which was partially followed Thorgaard's (1983) [5] statement where it was mentioned that the application of heat and cold shock (3 minutes after fertilization) resulted in decreased survival and production of triploidy rates. Such early shock may interfere to the fertilization process resulting, the production of diploid gynogens having reduced survival (Thorgaard, 1986) [15]. Alternatively hybrid catfish eggs may be more susceptible to direct damage from shock treatments at this time.

Na-Nakorn (1995) [13] made a comparison between cold shock and heat shock and showed that heat shock was more harmful and less effective than cold shock in inducing diplodization of walking catfish. But in this experiment, cold shock is more harmful to survival of eggs but more effective in triploidy induction. The reason behind this is unknown. Na-Nakorn (1995) [13] mentioned that may be the reason for the difference between shock and ambient temperature is a key factor determining the success of polyploid induction.

Hussain *et al*. (1991) [6] suggested that heat shock should be applied earlier than cold shock since it accelerated the rate of all biological process. In this experiment the highest survival rate was observed in heat shock which was prescribed by (Chourrout and Quillet, 1982) [17] where temperature-shock treatments were particularly advantageous for situations in which large volumes of eggs need to be treated. High proportions of triploid rainbow trout, Atlantic salmon (Benfey and Sutterlin, 1984) [19], Coho salmon, Chinook salmon and their hybrids have been produced with high survival after heat-shock treatments but in this experiment highest number of triploidy was found in cold shock treatment.

The negative effect of shock duration was also observed on *O. myskiss* (Chourrout, 1984) [14] and *C. idella* (Cassani and caton, 1985) [9]. In this experiment the high temperature with the duration more than 4 min caused the 100% mortality of eggs because eggs can probably support high temperature for a short time, but longer shock duration could apparently disrupt other cellular processes necessary for survival, as suggested by Cassani and Caton (1985) [9].

The hatching and survival percentage in cold shock treated groups were considerably lower than control group in this experiment which was similar to Gheyas *et al*. (2001) [20] where reported that hatching, survival and triploidy induction rates varied considerably among different lots of eggs subjected to the same cold shock. Ezaz *et al*. (1998) [21] reported similar in other species and have been attributed to factors such as egg quality differences or the susceptibility of eggs from different origins to shock treatments but the result obtained in this experiment from cold and heat shock was variable and proposed that the inconsistency might be for the size difference in eggs, smaller diameter of eggs might receive greater temperature shocks than larger diameter eggs within the same treatment batch. Hussain *et al*. (1991) [6] mentioned that time to start heat shock is critical. The same initiation time with different ambient temperatures prior to heat shock probably gave rise to different results. The lower hatching rate below 25 min duration of 6 $^o$C was due to the preponderance of aneuploid and mosaic embryos which was similar to Gheyas *et al*. (2001) [21]. In the case of hybrid catfish such factors in addition to egg quality could have been important.

To determine triploidy the flow cytometry is used in this experiment. Flow cytometry has a major emphasize in the quantity, accuracy and speed with which data were generated during this investigation. The use of flow cytometry is reliable and practical method to assess ploidy condition which was also proposed by Thorgaard *et al*. (1983) [5]. Flow cytometry used as a suitable techniques in rainbow trout, *Salmo gairdnari* Richardson (Solar *et al*., 1984) [22]. Ploidy determination can also be done by

chromosome counting but this method is labour intensive and requires the presence of actively dividing cells. Procedures for the measurement of erythrocyte volume are faster than chromosome counts but the results are less certain. This measurement focused on only indirect reflections of actual DNA content.

### 4.2 Growth performance

Fast *et al*. (1995) [8] reported that triploid are often sterile and lack of gonadal development, which can results in improved growth and food conversion, particularly when sexual maturity normally occurs before fish reach market size.

In this experiment growth of triploid was higher than control but lower than the shocked (6 $^{o}$C 25 min) diploid fry after sixteen weeks of rearing. Qin *et al*. (1998) [23] found that polyploid *Tilapia aurea* were larger than diploid control at 14 weeks.

In the genus *Clarias*, triploid *C. macrocephalus* were much larger than diploids at 8 months of age (Fast *et al*., 1995) [8]. Triploid *C. gariepinus* did not grow faster than diploids (Henken *et al*., 1987) [24]. Qin *et al*. (1998) [23] reported that *C. fuscus* were only 10% larger than diploid.

In this experiment triploid fish were15% larger than diploid control but lower than cold shocked diploid fry (27%). Qin *et al*. (1998) [23] also reported that use of all male triploid stocks may be desirable for *C. fuscus* commercial culture and could increase growth rate by 20% or more. The findings of this experiment further illustrate that three fish species of the same genus can exhibit markedly different growth rate in respect to triploidy and it highlights the necessity of triploidy effects on a species-specific basis.

Purdom (1972) [3] suggested that triplod plaice × flounder hybrids showed similar or better growth than diploid controls but Swarup (1959) cited from Purdom (1972) [3] reported no significant difference in growth between diploid and triploid sticklebacks. Lincoln (1981) cited from Purdom (1972) [3] concluded that the triploid hybrids of plaice × flounder did not show a growth advantage except for fillet weight, and Gervai *et al*. (1980) cited from Purdom (1972) [3] found no significant weight gain from triploidy in juvenile carps.

The slower growth rate in triploid hybrid catfish during early life may be offset by the subsequent potential benefits of sterility during later stages. Thorgaard (1986) [3] in rainbow trout; Wolters *et al*. (1982) [25] in channel catfish *Ictalurus punctatus* found the same result in early life cycle of the fishes.

Faster growth of triploid females has also been observed in loach, *Misgurnus anguillicaudatus* and rainbow trout *Oncorhynchus mykiss* after the normal age of sexual maturation (Thorgaard, 1986) [15]. Na-Nakorn (1999) [12] surmised that growth of gynogens was inferior over the control and prescribed that it might be the difference in culturing conditions.

The result of this experiment also similar to the results of Wolters *et al*. (1982) [25] reported that triploid channel catfish *Ictaluras punctatus* have a higher mean weight than diploid fish beyond the age of 8 months. However, all these growth benefits are not universal. Hussain *et al*. (1991) [6] reported that triploids of some species may exhibit inferior culture performance also. Thorgaard (1986) [15] reported that triploid common carp would not exhibit any growth advantage over diploids. These results variety depend on biological characteristics of a species, as well as on experimental conditions such as ambient temperature and feed quality (Thorgaard, 1986) [15].

Equal proportions of male and female were expected in triploids (Solar *et al*., 1984) [22] but it was revealed that histological observation of gonads explain a close to normal male-female ration in controls and a small excess of male (1:4:1) in the triploid fish. Sexual dimorphism in growth had been reported for several fishes of commercial interest (Hunter and Donaldson, 1983) [4]. Fast *et al*. (1995) [8] reported that female *Clarias macrocephalus* grew faster than male. Arai (2001) [16] reported that triploid males show better gonadal development. The original experiment was not designed for dimorphic growth study. Our findings clearly indicate that shocked diploid (may be the female was higher in this group) gave the higher growth than triploid which contrast markedly with *C. fuscus* where male fish grew significantly faster than females regardless of temperature, feed type, or ploidy.

In this study growth of shocked diploid fish was higher than the triploid. This is difficult to explain because other studies did not examine the growth of shocked diploid. Perhaps there were more female in this study. It would have been useful if the sex of the shocked diploids were examined. Argue *et al*. (2003) [26] reported that F1 hybrid *(Ictalurus punctatus* ×blue catfish, *Ictalurus furcatus*) female catfish had statistically higher fillet percentage.

In this experiment cold shock treated triploid and diploid has no significant survival difference. Several scientists (Fast *et al*., 1995) [8] reported different survival rate between cold treated triploid fish and diploid control but Qin *et al*. (1998) [23] found no difference in survival between diploid and triploid *C. fuscus*. The daily weight gain (DWG) in this experiment is higher in cold shocked diploid fish but Jamjun and Amara (2005) reported no significant differences in final BW, specific growth rate, survival rate, feed conversion ratio and protein efficiency ratio between diploid and triploid tilapia fish induce triploidy by using heat shock.

## 5. Conclusions and Recommendation

The experiment reported in this paper show that the tripod can be successfully induced in hybrid catfish through the use of cold shock rather than using the heat shock at proper condition. This work concludes that cold shock at 6 $^{o}$C 25 min applied to hybrid catfish eggs, give the highest triploidy rate and also cold shock longer than 30 min caused total mortality in hybrid catfish. The study suggests that the The effects of cold shock treatment on other cellular process require further study and also

observe the effect of temperature in 5, 7, 8 and 10 ºC in triploidy induction. Grow-out performance of triploidy hybrid catfish or the hybrid shocked diploid catfish could be determined to determine the utility for Aquaculture. FCR for triploid and cold shocked diploid fry can be measured in the next experiment

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