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Original Article

Effects of strains on growth performances of triploid bighead catfish, *Clarias macrocephalus* Günther, 1864

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ABSTRACT

Despite its importance, limited empirical information is available on the effects of parental strains on the performance of triploid aquatic animals, especially of tropical species. The present study was conducted to evaluate the impacts of strains on the growth performance of the triploid bighead catfish (*Clarias macrocephalus* Günther, 1864) which was produced using cold shock (7 °C, 25 min shock duration), applied to newly fertilized eggs obtained from all possible crosses between two catfish strains. The fingerlings of each group were reared until they were age 240 d. At age 60 d, the triploid fish had a lower specific growth rate (SGR), absolute growth rate (AGR), body length and body weight than the diploid counterparts. However, at harvest (age 240 d) body length and body weight were not different between the two groups while SGR of the cold shock group was higher than the diploid during age 121–240 d. The results revealed that parental strains did not show significant direct effects on the growth performance of triploids, but the interaction between paternal strain × shock was significant for the following traits: body length at age 90 d, body weight at age 90 d and 120 d, AGR for age 61–90 d, and SGR for age 61–90 d and 91–120 d. The interaction between paternal × maternal strain × shock was significant for body length, body weight at age 180 d and AGR for age 121–180 d. These results suggested that the growth performance of triploid *C. macrocephalus* cannot be enhanced by improving the parental strains; rather, improvement may be accomplished by selecting the parental strains based on triploid performance.

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Introduction

Triploidy is sometimes induced in fish to enhance the growth of the juvenile or post maturing individuals or both, making use of sterile animals; however, empirical data have shown variation in the results both among species and within a species (reviewed by Tiwary et al., 2004; Piferrer et al., 2009). Such variations are caused by various factors, namely culture conditions, for example: communal rearing with diploid (Cassani and Caton, 1986; Galbreath et al., 1994; O'Keefe and Benfey, 1997; Taylor et al., 2014) or rearing in lower oxygen supply (Hansen et al., 2015) suppressing growth of the triploid life stages resulting in pre- or post-maturation (Wolters et al., 1982; Ihssen et al., 1990; Brämick et al., 1995), before and after age 4 yr in sea bass, *Dicentrachus labrax* (Peruzzi et al., 2004). In addition, strains of the parent have been reported to affect the growth performance of triploid fish, for example: common carp, *Cyprinus carpio* (Taniguchi et al., 1986) and Atlantic salmon, *Salmo salar* (Sacobie et al., 2012), while interaction between family and ploidy was reported in salmonids, such as coho salmon, *Oncorhynchus kisutch* (Withler et al., 1998), rainbow trout, *Oncorhynchus mykiss* (Bonnet et al., 1999), brown trout, *Salmo trutta* Linnaeus, 1758 (Bonnet et al., 1999) and Atlantic salmon (Friars et al., 2001). Notably, no studies have been conducted with tropical fish.

Fish of the genus *Clarias* are of economic importance with global annual production exceeding 660,000 t and worth more than USD 970 million (as of 2013, FAO, 2015). Triploidy has been induced in various species of *Clarias* aiming at improving growth performance, but the results have varied considerably, for example: triploid < diploid (Na-Nakorn and Lakhaanantakun, 1993) and triploid > diploid (Fast et al., 1995) for *Clarias macrocephalus*; triploid > diploid for Chinese catfish, *Clarias fuscus* (Lacepède, 1803) (Qin et al., 1998) and triploid = diploid for *Clarias gariepinus* (Burchell, 1822) (Henken et al., 1987). To date, the causes of the discrepancy of the triploid *Clarias* performance have not been

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studied. Therefore, this study was conducted to investigate the effects of parental strains on the growth performance of triploid *C. macrocephalus*. The knowledge obtained will shed light on the possibility of improving the performance of triploids through strain selection based on diploid performance, not only in this species but also for other fish species in general.

Materials and methods

Experimental fish

Two strains of bighead catfish were used; the Department of Aquaculture strain (KU), which has been domesticated from wild fish from central and southern Thailand for more than 10 generations and the Udon-Thani strain (UD), which originated from wild fish in northeast Thailand and has been domesticated for about three generations. These two populations were genetically different as revealed by five microsatellite loci (Koolboon et al., 2014).

Artificial breeding and cold shock

Five gravid males (mean body weight 189.4 ± 3.3 g and 181.3 ± 3.1 g in the KU and UD groups, respectively) and females (mean body weight 215.9 ± 5.5 g and 209.9 ± 4.1 g in the KU and UD groups, respectively) were selected from each strain. The female fish were injected with 30 μg/kg LH-RH analogue (buserelin acetate) and 10 mg/kg dopamine antagonist (domperidone) while the dosage was 10 μg/kg LH-RH analogue and 10 mg/kg dopamine antagonist for male fish.

The female fish were stripped of eggs at about 16 h after injection. Prior to the stripping of the females, male fish were anesthetized and killed by spiking (Van de Vis et al., 2003), sacrificed and the testes removed. The testes were pooled within each strain, then cut into small pieces and squeezed in 30 mL of 0.6% normal saline.

Then, fertilization was performed within strains and between strains including reciprocal crosses resulting in four cross-types. In brief, 15 g of the pooled eggs from each strain were added with 2 mL pooled sperm solution from each strain and gently mixed. After thoroughly mixing, the eggs were activated with clean water at ambient temperature and immediately subjected to cold shock at 7 °C for 25 min (Na-Nakorn and Lakhaanantakun, 1993). In the control group, eggs without shock were incubated in hatching ponds supplied with water at ambient temperature (27 ± 2 °C). The hatching percentage of each group was determined.

Nursing of fry and rearing of experimental fish larvae

Nursing of the larvae from 7 d post hatching to age 30 d was performed in nursing tanks (1 m width, 2 m length and 1 m depth) with a water depth of 30 cm at a density of 1200 larvae per tank. They were fed *ad libitum* with water flea, *Moina* spp. in the first week and this diet was gradually changed to 40% protein artificial powdered feed and commercially available 35% protein walking catfish feed (Charoen Pokphand Feed Company Ltd.; Bangkok, Thailand) in the 4th week of rearing. Water quality was maintained by a 20% daily exchange of water.

Phase 1 growth trial

Fry aged 30 d from each group (4 cross-types × 2 treatments) were randomly transferred to four replicates in fiberglass tanks (1 m width, 1.5 m length, 0.6 m water depth) supplied with a flow-through water supply, at a stocking density of 500 fry per tank. They were fed to satiation with 35% protein walking catfish feed.

After 30 d of rearing, random samples of 50 fingerlings per replicate were taken for measurement of standard length and body weight. Then, the fingerlings from each tank were pooled within each group (cross-types × treatment) and used for the next growth trial.

Phase 2 growth trial

Before starting, the experimental fish were subjected to grading to remove outliers. Then, random samples (n = 200 individuals/cross-type) were taken from each cross-type. No difference was found between the means of the individual body weight of the samples before and after grading (p = 0.205–0.945, Student's t test). The fingerlings were stocked into four replicate rearing tanks (1 m width, 2 m length and 1 m depth) with a water depth of 0.8 m at 75 individuals per tank. They were fed *ad libitum* with 30% protein walking catfish feed (Charoen Pokphand Feed Company Ltd.; Bangkok, Thailand) until 16 wk and then this was changed to the 25% protein feed of the same brand. Water quality was maintained by exchanging 20% of the water once a week.

At age 90 d, 120 d, 180 d and 240 d, the standard length and weight were measured in random samples of 25 individuals per tank. The fish were harvested at age 240 d and the survivors were counted and a sample of 25 fish per tank was randomly taken, sacrificed and individual gonads were weighed. Sexes were identified according to the appearances of the gonads into three categories; males, females, and unidentified group wherein the last category of gonad was a thread-like, thin, and transparent strip. Gonadosomatic indices were calculated as the percentage of gonad weight by body weight.

The absolute growth rate [AGR; Equation (1)] and specific growth rate [SGR; (Equation (2)] for weight were calculated:

$$AGR_w = \frac{W_f - W_i}{T_f - T_i} \quad (1)$$

$$SGR_W = \frac{\ln W_f - \ln W_i}{T_f - T_i} \times 100 \quad (2)$$

where W_i = initial weight, W_f = final weight, T_i = initial age and T_f = final age (Jobling, 1983).

The condition factor (K) was calculated using Equation (3):

$$K = \frac{100 \times W}{L^3} \quad (3)$$

where W = body weight and L = standard length (Htun-Han, 1978).

The gonadosomatic index (GSI) was calculated using Equation (4):

$$GSI = \frac{W_g}{W_b} \times 100 \quad (4)$$

where W_g = gonad weight and W_b = body weight (Dahlgren, 1979).

Ploidy evaluation

Ploidy evaluation was performed at age 3 d using a chromosome count which involving 30 larvae per treatment (30 metaphase/individual) using the applied protocol of Nanda et al. (1995).

Data analyses

The data of each trait (hatching rates, success rate or percentage of triploidy in each cross-type; body weight, total length, absolute growth rate, specific growth rate) at age 60 d were analyzed using ANOVA following Equation (5):

$$y = \mu + S + D + shock + (S \times D) + (S \times shock) + (D \times shock) + (S \times D \times shock) + e \quad (5)$$

where y = mean of trait, μ = constant, S = sire (male) strain, D = dam (female) strain, shock = treatments (control or cold-shocked), S × D = interaction between sire and dam, S × shock = interaction between sire and treatments, D × shock = interaction between dam and treatments, S × D × shock = interaction between sire, dam and treatments and e = error.

The data of the same traits measured at age 90 d, 120 d, 180 d and 240 d (survival rates, condition factor and gonadosomatic indices of each sex at harvest) were analyzed using the above model with survival rates at harvest included in the model. The survival rate was included in the model because in the preliminary data analysis, a significant difference between the cold-shocked and the control groups was found for the survival rate. The difference might affect the culture density and thus also affect growth performance (Hossain et al., 1998; Toko et al., 2007). The sex ratios at harvest were tested against the 1:1 ratio using the χ^2 test.

Prior to the analyses, all datasets were tested for normal distribution; only the survival rates at age 120 d, 180 d and 240 d were not normally distributed. Therefore, they were transformed using the arc-sin transformation.

Results

Hatching percentage and success rate of triploid induction

The hatching rates of the cold-shocked eggs were low (26.55 ± 7.45% to 38.92 ± 5.58%) and were not significantly different between cross-types but they were significantly ($p < 0.01$) lower than those of the control groups (75.19 ± 7.14 to 79.46 ± 9.71%) as shown in Table 1. ANOVA revealed significant ($F = 221.61$, $p < 0.0001$) effects of treatments (cold shock) on hatching rates while the other factors, parental strains, interaction between paternal, maternal strains and treatments, and interaction between each parental strain and treatments did not show any significant effects on this trait.

Chromosome counts showed that the triploid fish had 81 chromosomes while the diploid fish had 54 chromosomes. Success rates were between 83.33% and 96.67% (average = 90.00 ± 6.09%) and not different among the four cross-types (Table 1).

Growth between age 30–60 d (in fiberglass tanks)

At age 60 d, the cold-shocked group (body length, BL = 5.05 ± 1.42 cm and body weight, BW = 1.39 ± 1.19 g) was significantly ($F_{BL} = 5.47$, $p = 0.03$; $F_{BW} = 7.58$, $p = 0.01$) smaller than the control (BL = 5.53 ± 1.35 cm; BW = 1.79 ± 1.32 g) as shown in Table 2. The same trend was seen for AGR and SGR, where those of the cold-shocked groups (AGR = 0.03 ± 0.01 g/d; SGR = 4.88 ± 0.63%/d) were significantly ($F_{AGR} = 7.31$, $p = 0.01$; $F_{SGR} = 7.69$, $p = 0.01$) lower than those of the control groups (AGR = 0.04 ± 0.01 g/d; SGR = 5.48 ± 0.47%/d).

Interaction between factors, maternal, paternal strains and treatments did not have any significant effects on length (BL), weight (BW), AGR and SGR of the experimental fish with values of F_{BL} = 0.00–0.40, p = 0.53–0.98; F_{BW} = 0.00–0.85, p = 0.37–0.98; F_{AGR} = 0.04–0.42, p = 0.52–0.84; F_{SGR} = 0.05–0.24, p = 0.63–0.82, respectively.

Performance at age 61–240 d

Survival rates at age 240 d

Survival rates (Table 3) of the cold-shocked groups (average = 82.00 ± 3.50%) were lower ($F = 36.08$, $p < 0.01$) than that of the control (average = 88.67 ± 6.00%). It should be noted that, among the cold-shocked groups, the survival rate of UD × KU was significantly lower than the others. Among the studied factors, only cold shock and interaction between paternal strains × cold shock showed significant ($F = 7.82$, $p < 0.05$) effects on survival rates.

Body weight, length, absolute growth rate and specific growth rate

BW and BL from age 90–240 d are shown in Table 4. At harvest, the cold shocked and the control groups had average BW and BL values of 105.42 ± 31.29 g; 22.92 ± 2.22 cm and 109.09 ± 38.18 g; 23.18 ± 2.70 cm, respectively. Both growth rate parameters, AGR and SGR were high in the early stages and gradually decreased as the fish grew up (Table 4).

Effects of strains on growth parameters

Body length and body weight

Strain had no significant effects on BW (F = 0.02–4.76, p = 0.06–0.84) and BL (F = 0.11–5.35, p = 0.07–0.79) throughout the study period. However, the strain of the male parent interacting with treatments showed a significant effect on BL at age 90 d ($F = 12.24$, $p < 0.01$) and on BW at age 90 d ($F = 10.90$, $p < 0.01$) and at age 120 d ($F = 4.46$, $p = 0.04$). Similarly, at age 180 d, the interaction between paternal × maternal strains × treatment also had a significant effect on BL ($F = 4.76$, $p = 0.04$) and BW ($F = 5.86$, $p = 0.03$).

Absolute growth rate based on weight

Strains had no significant effects on AGR (F = 0.08–5.86, p = 0.08–0.99). The interaction between paternal strains × cold shock had significant ($F = 11.71$, $p < 0.01$) effects on this trait during age 61–90 d, while interactions between maternal strains × cold shock and paternal × maternal strains did not have effects on AGR (F = 0.20–0.79, p = 0.38–0.66; F = 0.08–3.90, p = 0.06–0.78, respectively) except for a significant ($F = 5.22$, $p = 0.03$) interaction between paternal × maternal strains × treatments on AGR during age 121–180 d.

Specific growth rate based on weight

The effects of strains and all combinations of interaction between paternal strains, maternal strains and cold shock on SGR were not significant (F = 0.29–3.90, p = 0.06–0.92), except for the interaction between paternal strain × cold shock on SGR during age 61–90 d ($F = 7.23$, $p = 0.01$) and during age 91–120 d ($F = 6.44$, $p = 0.02$).

Table 1
Mean (±SD) hatching percentages of control and cold-shocked groups using brooders from Kasetsart University strain (KU) and Udon-Thani strain (UD) of *Clarias macrocephalus* and percentage of triploid offspring observed in the cold-shocked groups.

Crosses (Sire × Dam)	Hatching (%)		Triploid offspring† (%)
	Control	Cold-shocked	
KU × KU	75.19 ± 7.14	28.29 ± 5.79	93.33
KU × UD	78.95 ± 2.89	29.34 ± 3.48	86.67
UD × KU	76.88 ± 13.76	38.92 ± 5.58	96.67
UD × UD	79.46 ± 9.71	26.55 ± 7.45	83.33
Mean	77.62 ± 8.09^{A*}	30.78 ± 7.02^{B}	90.00 ± 6.09

* = different upper case superscripts indicate significant ($p < 0.01$) difference between control and cold-shocked groups.
† = percentage of triploids were determined in pooled samples from three replications of each treatment.

Table 2
Mean (±SD) of body length (BL), body weight (BW) at age 60 d, absolute growth rate (AGR) and specific growth rate (SGR) during age 30–60 d of the control and cold-shocked groups using brooders from Kasetsart University strain (KU) and Udon-Thani strain (UD) of *Clarias macrocephalus*.

Cross (Sire × Dam)	BL (cm)		BW (g)		AGR (g/d)		SGR (%/d)	
	Control	Cold-shock	Control	Cold-shock	Control	Cold-shock	Control	Cold-shock
KU × KU	5.36 ± 1.13	4.90 ± 1.37	1.71 ± 1.08	1.36 ± 1.20	0.03 ± 0.01	0.02 ± 0.01	5.33 ± 0.39	4.86 ± 0.57
KU × UD	5.93 ± 1.42	5.20 ± 1.39	2.06 ± 1.43	1.42 ± 1.12	0.04 ± 0.01	0.03 ± 0.01	5.68 ± 0.36	4.88 ± 0.80
UD × KU	5.87 ± 1.39	5.34 ± 1.52	2.11 ± 1.49	1.61 ± 1.33	0.04 ± 0.00	0.03 ± 0.01	5.74 ± 0.21	5.17 ± 0.65
UD × UD	4.92 ± 1.21	4.62 ± 1.28	1.23 ± 1.02	0.99 ± 0.92	0.03 ± 0.01	0.02 ± 0.00	4.81 ± 0.73	4.32 ± 0.03
Mean	5.53 ± 1.35^{A}	5.05 ± 1.42^{B}	1.79 ± 1.32^{A}	1.39 ± 1.19^{B}	0.04 ± 0.01^{A}	0.03 ± 0.01^{B}	5.48 ± 0.47^{A}	4.88 ± 0.63^{B}

Note: different upper case superscripts indicate significant ($p < 0.01$) difference between control and cold-shocked groups.

Table 3
Survival rate of the control and cold-shocked groups using brooders from Kasetsart University strain (KU) and Udon-Thani strain (UD) of *Clarias macrocephalus*.

Crosses	Survival rate (%)	
	Control	Cold shock
KU × KU	83.00 ± 3.98^{C}	82.33 ± 1.68^{A}
KU × UD	91.33 ± 2.31^{AB}	83.00 ± 1.68^{A}
UD × KU	96.67 ± 0.94^{A}	76.00 ± 5.66^{B}
UD × UD	87.67 ± 6.20^{BC}	83.67 ± 2.75^{A}
Mean	88.67 ± 6.00^{X}	82.00 ± 3.50^{Y}

Note: different uppercase superscripts in the same column denote significant difference ($p < 0.05$); different uppercase superscripts for the row of means indicate a significant difference ($p < 0.01$).

Effects of cold-shock

Growth parameters

Cold-shock had no significant effects on BL ($F = 0.25–2.94$, $p = 0.10–0.62$), BW ($F = 0.14–2.82$, $p = 0.11–0.71$) and AGR ($F = 0–2.35$, $p = 0.07–0.98$) at all stages. The results of SGR were slightly different in that cold shock resulted in a significant ($F = 4.82$, $p = 0.04$) difference of SGR during age 181–240 d, when the cold-shocked groups showed higher SGR values than that of the control (SGR = 0.28 ± 0.08%/d and 0.38 ± 0.09%/d in the control and cold-shocked group, respectively).

Condition factor

The condition factor (K) of the control and cold-shocked groups fell in the same range (K = 0.80–0.85). Neither the factors nor interactions between them had significant effects on K value ($F = 0.03–7.94$, $p = 0.06–0.59$).

Sex ratios and gonadosomatic index

At harvest, the cold-shocked individuals differentiated into males and females with sex ratios in most cross-types not significantly ($p = 0.10–0.27$, χ^2-test) different from the 1:1 ratio (female:male ranged from 1:1.63 to 1:5) except for the KU × KU cold-shocked group for which the sex ratio deviated from 1:1 toward maleness ($p = 0.003$, χ^2-test). In the control group, sex ratios

Table 4
Mean (±SD) of body length (BL), body weight (BW), absolute growth rate (AGR), specific growth rate (SGR), condition factor (K) of offspring of four crosses of *Clarias macrocephalus* from Kasetsart University strain (KU) and Udon-Thani strain (UD) for cold-shocked and control groups at ages 90 d, 120 d, 180 d and 240 d.

Parental strains	90 d		120 d		180 d		240 d	
	Control	Cold-shock	Control	Cold-shock	Control	Cold-shock	Control	Cold-shock
BL (cm)								
KU × KU	17.37 ± 1.90	14.39 ± 2.95	19.94 ± 2.34	18.58 ± 2.69	22.99 ± 1.84	21.72 ± 3.02	23.89 ± 2.05	23.44 ± 2.64
KU × UD	18.28 ± 3.58	15.16 ± 2.25	19.45 ± 2.40	18.61 ± 2.56	22.65 ± 2.12	22.02 ± 2.15	23.50 ± 1.90	23.38 ± 2.46
UD × KU	16.92 ± 1.60	15.85 ± 2.41	19.41 ± 2.12	19.35 ± 2.38	21.36 ± 1.81	22.60 ± 2.31	22.07 ± 1.97	23.70 ± 2.60
UD × UD	16.10 ± 1.77	14.10 ± 2.09	17.98 ± 2.37	17.30 ± 2.66	20.99 ± 1.70	20.17 ± 2.14	21.57 ± 2.36	21.26 ± 2.73
Mean	17.32 ± 2.52^{A}	14.99 ± 2.57^{B}	19.72 ± 2.08^{A}	18.61 ± 2.63^{B}	22.14 ± 2.0^{A}	21.84 ± 2.58^{A}	22.92 ± 2.22^{A}	23.18 ± 2.70^{A}
BW (g)								
KU × KU	46.59 ± 13.69	28.97 ± 18.28	67.53 ± 22.10	56.93 ± 22.62	97.83 ± 23.46	84.95 ± 34.09	117.63 ± 31.75	110.91 ± 38.21
KU × UD	49.79 ± 11.89	30.23 ± 13.42	62.51 ± 21.06	55.92 ± 21.77	94.16 ± 25.02	86.36 ± 26.50	110.24 ± 29.16	108.19 ± 33.92
UD × KU	43.16 ± 12.30	37.90 ± 16.60	63.78 ± 19.35	64.84 ± 22.15	81.60 ± 23.18	98.71 ± 29.02	96.21 ± 27.94	120.69 ± 39.64
UD × UD	35.60 ± 11.63	24.83 ± 11.81	50.73 ± 18.02	45.69 ± 18.69	76.02 ± 20.94	66.80 ± 22.91	89.78 ± 29.45	84.04 ± 31.84
Mean	44.96 ± 13.27^{A}	31.35 ± 16.28^{B}	64.94 ± 19.08^{A}	57.16 ± 22.44^{B}	89.00 ± 24.85^{A}	86.69 ± 30.67^{A}	105.42 ± 31.29^{A}	109.09 ± 38.18^{A}
AGR (g/d)								
KU × KU	1.50 ± 0.21	0.92 ± 0.36	0.70 ± 0.14	0.93 ± 0.09	0.51 ± 0.09	0.47 ± 0.11	0.32 ± 0.12	0.43 ± 0.18
KU × UD	1.59 ± 0.09	0.98 ± 0.17	0.64 ± 0.12	0.86 ± 0.25	0.42 ± 0.13	0.51 ± 0.11	0.27 ± 0.06	0.36 ± 0.07
UD × KU	1.37 ± 0.07	1.21 ± 0.22	0.65 ± 0.09	0.89 ± 0.26	0.31 ± 0.16	0.57 ± 0.13	0.24 ± 0.04	0.37 ± 0.15
UD × UD	1.42 ± 0.25	0.79 ± 0.14	0.67 ± 0.03	0.70 ± 0.09	0.34 ± 0.06	0.35 ± 0.11	0.23 ± 0.08	0.29 ± 0.02
Mean	1.44 ± 0.20^{A}	0.99 ± 0.27^{B}	0.67 ± 0.10^{B}	0.87 ± 0.19^{A}	0.40 ± 0.14^{A}	0.49 ± 0.12^{A}	0.27 ± 0.08^{B}	0.37 ± 0.13^{A}
SGR (%/d)								
KU × KU	11.05 ± 0.82	10.13 ± 1.50	1.25 ± 0.29	2.39 ± 0.71	0.63 ± 0.12	0.67 ± 0.15	0.30 ± 0.12	0.43 ± 0.12
KU × UD	10.68 ± 0.74	10.37 ± 1.45	1.09 ± 0.22	2.03 ± 0.36	0.51 ± 0.15	0.75 ± 0.25	0.26 ± 0.06	0.38 ± 0.08
UD × KU	10.07 ± 0.42	10.70 ± 0.84	1.24 ± 0.14	1.82 ± 0.59	0.44 ± 0.21	0.70 ± 0.18	0.28 ± 0.06	0.33 ± 0.13
UD × UD	11.07 ± 1.97	10.70 ± 0.62	1.52 ± 0.29	2.06 ± 0.07	0.52 ± 0.13	0.64 ± 0.25	0.27 ± 0.08	0.38 ± 0.02
Mean	10.67 ± 0.89^{A}	10.44 ± 1.12^{A}	1.24 ± 0.25^{B}	2.08 ± 0.53^{A}	0.53 ± 0.16^{B}	0.69 ± 0.18^{A}	0.28 ± 0.08^{B}	0.38 ± 0.09^{A}
K								
KU × KU	0.87 ± 0.20	0.85 ± 0.16	0.82 ± 0.08	0.84 ± 0.08	0.79 ± 0.09	0.78 ± 0.07	0.84 ± 0.09	0.83 ± 0.10
KU × UD	0.84 ± 0.11	0.82 ± 0.09	0.81 ± 0.09	0.82 ± 0.06	0.80 ± 0.10	0.78 ± 0.09	0.83 ± 0.07	0.81 ± 0.07
UD × KU	0.87 ± 0.07	0.88 ± 0.09	0.83 ± 0.08	0.86 ± 0.09	0.82 ± 0.09	0.84 ± 0.14	0.87 ± 0.08	0.87 ± 0.09
UD × UD	0.82 ± 0.06	0.82 ± 0.11	0.84 ± 0.06	0.82 ± 0.06	0.80 ± 0.07	0.78 ± 0.08	0.86 ± 0.09	0.84 ± 0.10
Mean	0.85 ± 0.13^{A}	0.85 ± 0.12^{A}	0.82 ± 0.08^{A}	0.84 ± 0.08^{A}	0.80 ± 0.09^{A}	0.80 ± 0.10^{A}	0.85 ± 0.08^{A}	0.84 ± 0.09^{A}

Note: different uppercase superscript letters denote a significant ($p < 0.01$) difference between means of cold-shocked and control groups at each age.

Table 5
Sex ratio and mean gonadosomatic index (GSI; ±SD) of male, female and unidentified sex offspring averaged within each cross of the cold-shocked and control groups of four crosses of Clarias macrocephalus from Kasetsart University strain (KU) and Udon-Thani strain (UD).

Crosses	Sex ratio (female: male)		Unidentified (out of $n = 35$)		GSI-male		GSI-female		GSI-unidentified	
	Control	Cold-shock	Control	Cold-shock	Control	Cold-shock	Control	Cold-shock	Control	Cold-shock
KU × KU	1:1.82	1:5.33	4	16	0.64 ± 0.31	0.41 ± 0.16	7.31 ± 2.20	0.45 ± 0.30	0.36 ± 0.33	0.26 ± 0.25
KU × UD	1:0.83	1:1.63	2	14	0.52 ± 0.19	0.48 ± 0.21	7.85 ± 2.90	0.32 ± 0.25	0.38 ± 0.17	0.18 ± 0.12
UD × KU	1:0.67	1:5.0	2	25	0.56 ± 0.20	0.32 ± 0.26	6.70 ± 2.70	1.76 ± 2.93	0.23 ± 0.19	0.33 ± 0.22
UD × UD	1:0.47	1:2.33	3	29	0.55 ± 0.16	0.48 ± 0.23	7.15 ± 2.95	0.52 ± 0.00	0.09 ± 0.03	0.25 ± 0.20
Mean	1:0.95	1:3.57	2.7 ± 0.96	21.0 ± 7.16	0.57 ± 0.23^{A}	0.42 ± 0.20^{B}	7.20 ± 2.72^{A}	0.65 ± 1.27^{B}	0.27 ± 0.27^{A}	0.26 ± 0.21^{A}

Note: mean values within the cold-shocked or control group with different uppercase superscripted letters are significantly ($p < 0.01$) different.

ranged from 1:0.47 to 1:1.82 in each cross-type (Table 5) and did not significantly ($p = 0.07–0.60$, χ^2-test) deviate from 1:1. Notably, there were individuals without sex differentiation in both the control and the cold-shocked groups, with the cold-shocked group showing a higher incidence of undifferentiated individuals (21.0 ± 7.16 individuals), compared to 2.7 ± 0.96 individuals in the control groups (n = 35).

The GSI of the control females was 7.20 ± 2.72, while the male GSI was 0.57 ± 0.23, which was significantly higher ($F_{female} = 49.26$, $p < 0.01$; $F_{male} = 8.78$, $p < 0.01$) than that of the respective cold-shocked group (female GSI = 0.65 ± 1.27; male GSI = 0.42 ± 0.20). The undifferentiated individuals did not show a significant ($F = 0.35$, $p = 0.56$) difference in the GSI between the cold-shocked and control groups (0.26 ± 0.21and 0.27 ± 0.27, respectively).

Discussion

Effects of strains on traits of triploid

The results demonstrated that the strains of the sire or dam did not have a direct effect on the traits of the triploid, while the strains of the sire showed a significant interaction with cold-shock on growth traits (BL at age 90 d, BW at age 90 d and 120 d, AGR during age 61–90 d and SGR during age 61–90 d and age 91–120 d) of the triploid. This implies that the average performances of the diploid crosses sired by each strain did not proportionately predict the average performances of the triploid counterpart. On the contrary, interaction between the strains of the dams and treatment was not significant, while a significant interaction between the strains of the sire × strains of dam × treatment was shown for BL, BW, and AGR at age 180 d. The present results were in agreement with previous reports which supported the interaction between ploidy and strains/families; for example, the effect of ploidy × family on BW of small size (8.5 g), SGR of pre- and post smolt coho salmon (Withler et al., 1998); on BW, BL and condition factor of small size rainbow trout and brown trout (Bonnet et al., 1999); male × female × shock effect on levels of plasma *IGF-1*of triploid ocean-type chinook salmon, *Oncorhynchus tshawytscha* smolts (Shrimpton et al., 2007); the sire × ploidy effect on weight and Na^+-K^+ ATPase and the dam × ploidy effect on SGR for weight in ocean type chinook salmon (Shrimpton et al., 2012). Similarly, Friars et al. (2001) indirectly reported such interaction by showing a not significant linear regression of least square mean BL and BW of diploid on those of triploid Atlantic salmon. Furthermore, the effects of parental strains on the performance of triploids relative to the diploid counterparts were reported in fancy carp (Taniguchi et al., 1986). In addition, although the experimental design did not allow for assessment of such interaction, Sacobie et al. (2012) showed differential growth of triploid arising from different strains of Atlantic salmon.

The present study revealed significant effects of paternal strains (through interaction with ploidy), but not maternal strains, on the growth performance of triploids. This finding was rather unexpected owing to the fact that triploids possess two maternal genomes and only one paternal genome. The present result may be explained by the gene expression studies in triploids where the expression level of triploid genes was not always dose-dependent (Suzuki et al., 1999; Pala et al, 2008; Devlin et al., 2014). Therefore, it is not necessary that the two maternal genomes would dominate a single paternal genome.

Notably, supportive evidence for the predominance of dam effects has also been reported where a quantitative genetics approach showed significant variance of the triploid phenotype due to the dam component (Blanc et al., 2005; Shrimpton et al., 2012) in two salmonid species despite significant sire × ploidy effects in some traits (Shrimpton et al., 2012). The discrepancies in the results may be because of the difference in species and genetic background of the fish and thus require further study.

Effects of ploidy on traits of triploid

Growth

The present results on the growth of triploid bighead catfish, regardless of strains, do not agree with previous reports in the same species where the growth of triploids was either inferior (Na-Nakorn and Lakhaanantakun, 1993) or superior to that of diploids (Fast et al., 1995). The discrepancy in the results may be explained by the difference in the parental strains as shown in the present study and those of Taniguchi et al. (1986), Withler et al. (1998) and Bonnet et al. (1999). Furthermore, different rearing conditions may also impact the results. For example, communal rearing of diploids and triploids impaired the growth of triploids in many species, such as grass carp, *Ctenopharyngodon idella* (Cassani and Caton, 1986) and Atlantic salmon (Galbreath et al., 1994; O'Keefe and Benfey, 1997; Taylor et al., 2014). Therefore, the presence of different percentages of diploids in the so-called triploid group (20% diploid for Na-Nakorn and Lakhaanantakun (1993); 10% for the present study and 4% for that of Fast et al. (1995)) may be partly responsible for the discrepancy in the growth comparison results.

Survival rates

The present results on the survival rate (2n > 3n) corroborated the results of Na-Nakorn and Lakhaanantakun (1993), while the report of Fast et al. (1995) on the same species did not mention this trait. Different trends in triploid survival rates have been reported in *C. gariepinus* (2n = 3n) by Karami et al. (2010) and other Silurid fishes, namely, 2n > 3n in far eastern catfish, *Silurus asotus* (Nam et al., 2001). Decreases in triploid survival rates relative to diploids were also reported in other fish taxa such as Atlantic salmon during salt water rearing (Galbreath and Thorgaard, 1995) and triploid hybrid red tilapia, *Oreochromis mossambicus* × *Oreochromis*

niloticus (Pradeep et al., 2012). The underlying reason for this is still not clear. However, a few studies reported some defects of triploid that may hamper the ability to survive, for example: reduce the gill area of triploid Atlantic salmon might hamper normal respiration (Sadler et al., 2001); lower the ability of triploid shi drum, *Umbrina cirrosa* to cope with stress as indicated by haematological parameters (Ballarin et al., 2004); produce a less efficient respiratory apparatus in triploid stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) (Tiwary and Ray, 2004); and lower the tolerance to suboptimal rearing condition of Atlantic salmon, such as high temperature and hypoxia (Hansen et al., 2015). Similar defects could have occurred in the triploid fish in the present study and lowered the survival rate.

Condition factor

In the present study, neither strains nor treatments had significant effects on the condition factor of triploids and diploids. In most species, the condition factors of diploid and triploid are not different except for a few species, namely Atlantic salmon (Galbreath and Thorgaard, 1995; Benfey, 2001; Sacobie et al., 2012; Taylor et al., 2014), rainbow trout (Withler et al., 1998; Bonnet et al., 1999; Friar et al., 2001), brown trout (Blanc et al., 2005) and ocean-type chinook salmon (Shrimpton et al., 2007), where 2n > 3n with some exceptions.

Sterility of triploid

Neither parental strains nor interaction between the sire, dam and treatment affected the sterility of triploids, while treatments (ploidy) did. Both male and female triploids had a lower GSI than that of the diploid counterpart, indicating sterility of triploids. Sterility of the triploid is common and has been reported in all triploid cases including fishes in the Order Siluriformes, such as bighead catfish (Na-Nakorn and Lakhaanantakun, 1993; Fast et al., 1995), European catfish (Krasznai and Marian, 1986), Indian catfish (Tiwary et al., 1997) and Chinese catfish (Qin et al., 1998).

Sex ratio

The sex ratio of one triploid group skewed toward maleness while the number of individuals with an unidentified sex increased. A skewed sex ratio of the triploid relative to the diploid is not common although it has been reported such as toward femaleness in turbot, *Scophthalmus maximus* (Cal et al., 2006), *Penaeus monodon* (Pongtippatee et al., 2012) and *Penaeus merguiensis* (Hidayah et al., 2013) and toward maleness in red tilapia, *O. mossambicus* × *O. niloticus* (Pradeep et al., 2012). The sex ratio of the triploid may vary, with the sex determining system of that species. Bighead catfish follows the female homogamety sex determining system (Na-Nakorn, 1995), and thus the sex ratio of the triploid, which was not reported in previous studies (Na-Nakorn and Lakhaanantakun, 1993; Fast et al., 1995), should be 1:1. This may imply that female triploid individuals with severe gonadal retardants were included in the unidentified sex group and thus reduced the number of female triploids.

The study showed that the overall growth performance of triploid bighead catfish was not different from those of the diploid. The strains of the sire have significant interaction with ploidy in determining the growth of the triploid while the strains of the dam did not have significant effects. Therefore, it is not possible to improve the growth of the triploid by selection of good performance diploids; rather, the selection should be done directly on triploid performance. In addition, triploid bighead catfish may not be suitable for aquaculture because they did not show superiority in growth over the diploid, while the survival rate was low. However, triploids are useful for basic studies to understand the mechanisms controlling growth and reproduction.

Conflict of interest

The authors state that there is no conflict of interest.

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