Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 276 (2008) 50–59
Aquaculture
www.elsevier.com/locate/aqua-online

The effects of triploidy on *Penaeus* (*Marsupenaeus*) *japonicus* (Bate) survival, growth and gender when compared to diploid siblings

Frank E. Coman [a,*], Melony J. Sellars [a], Belinda J. Norris [b], Greg J. Coman [a], Nigel P. Preston [a]

[a] *CSIRO Marine and Atmospheric Research, 233 Middle Street, Cleveland, Qld 4163, Australia*
[b] *CSIRO Livestock Industries, Queensland Biosciences Precinct, 306 Carmody Road, St Lucia, Qld 4067, Australia*

Received 10 May 2007; received in revised form 17 January 2008; accepted 20 January 2008

Abstract

In this study the effect of triploidy on survival, growth and gender of the Kuruma shrimp, *Penaeus* (*Marsupenaeus*) *japonicus* (Bate), was assessed by making comparisons to diploid sibling performance. Triploidy was induced by preventing polar body II extrusion using 6-dimethylaminopurine (6-DMAP) chemical shock. Four families with a triploid induction rate greater than 70% were reared for approximately 300 days (10 months), during which time their survival, growth, gender ratios and individual ploidy level were assessed. All triploids were found to be female resulting in significantly skewed gender ratios. Males treated with 6-DMAP were either diploid or mosaic (having both diploid and triploid cell populations). 6-DMAP treated shrimp in families 1 and 2 had significantly poorer ($P<0.05$) survival performance from PL10 to tagging size (PL187) compared to controls. Survival of family 4 control animals from PL10 to tagging size (PL118) was also significantly greater than the treatment animals, however, there was no difference in the survival performance of control and 6-DMAP treated shrimp for family 3 ($P>0.05$) over the same period. Similarly, for all four families, survival of 6-DMAP treated and control shrimp was not significantly different ($P>0.05$) from tagging through to assessment age (PL251 for families 1 and 2, and PL173 for families 3 and 4). Treatment with 6-DMAP had a significantly negative effect on weight of females at assessment age when compared to controls of families 1, 2 and 3, and males of families 1 and 4. Diploid females that had received treatment with 6-DMAP were significantly heavier ($P<0.05$) than triploid females for family 3 at assessment age, and family 4 from assessment age through to the end of the rearing period. The discovery that all triploid *P. japonicus* are female could be of significant commercial interest in the future, as shrimp growth rates are sexually dimorphic with females growing faster and larger than males. However, triploidy induced by 6-DMAP chemical shock appears to have detrimental effects on growth and survival performance from sub-adult to reproductive size when compared to diploid siblings. The production of only female triploids supports the hypothesis that the female is the heterogametic gender for *P. japonicus*.
Crown Copyright © 2008 Published by Elsevier B.V. All rights reserved.

Keywords: Kuruma shrimp; *Penaeus japonicus*; Prawn; Genetic protection

1. Introduction

In Australia *Penaeus japonicus* (Bate) is the highest value farmed shrimp, produced exclusively for live export (Hewitt and Duncan, 2001). Successful genetic improvement of Australian strains of *P. japonicus* has prompted the search for techniques to prevent unauthorised breeding from these elite genotypes. The production of sterile shrimp for pond growout has the potential to protect these living genetic resources whilst also having the possibility of conferring other commercially desirable traits.

There are many reported techniques for conferring sterility in invertebrates and fish including polyploidy, interspecies hybridization, hormone treatment, irradiation and regulation of gene expression. Of these, triploidy is the most widely documented method attempted for inducing sterility with successful reports for shrimp (Li et al., 1999, 2003a,b; Zhang

* Corresponding author. Tel.: +61 7 3826 7357; fax: +61 7 3826 7222.
E-mail address: Frank.Coman@csiro.au (F.E. Coman).

0044-8486/$ - see front matter. Crown Copyright © 2008 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.01.031

et al., 2003, 2004; Norris et al., 2005; Sellars et al., 2006; Xiang et al., 2006), abalone (Stepto and Cook, 1998; Zhang et al., 1998), oysters (Stanley et al., 1981; Guo and Allen, 1994), clams (Vadopalas and Davis, 1998) and mud loaches (Nam et al., 2004). Triploid animals are currently being grown on a commercial scale for various species including Eastern oysters (Nell, 2002), Sydney rock oysters (Hand et al., 2004), scallops (Heasman et al., 1998) and several fish species (Tave, 1993).

In addition to conferring sterility, triploid animals of various cultured species have been found to have other commercial benefits. In some instances triploids have higher growth rates (Beaumont and Fairbrother, 1991; Benfey, 1999; Brake et al., 2004; Xiang et al., 2006), skewed gender ratios (Li et al., 2003b) and improved pathogen resistance (Peyre et al., 1999) compared with diploid stocks. Triploid induction techniques have been developed for a number of penaeid species (*Penaeus* (*Fenneropenaeus*) *chinensis*, Bao et al., 1994; Lin and Cai, 1996; Li et al., 1999, 2003a,b; Zhang et al., 2003, 2004; Xiang et al., 2006; *Penaeus* (*Litopennaeus*) *vannamei*, Dumas and Ramos, 1999; *P. japonicus*, Norris et al., 2005; Sellars et al., 2006), however, the only reports of the effect of triploidy on shrimp performance are from Li et al. (1999, 2003b) and Xiang et al. (2006) of the Institute of Oceanology at the Chinese Academy of Sciences. They reported that female triploid *P. chinensis* had abnormally developed ovaries and were sterile. Li et al. (2003b) also reported gender ratios skewed towards females for triploid *P. chinensis* and that triploid testes were less developed and had less sperm than diploid testes. Apart from the findings of this Chinese research team, there have been no definitive reports or detailed studies completed on the comparative survival and growth performance, or gender ratio of triploid versus diploid sibling penaeid shrimp to establish their suitability for commercial scale production.

This study uses protocols outlined by Norris et al. (2005) to produce four triploid *P. japonicus* families using 6-dimethylaminopurine (6-DMAP) to prevent polar body II extrusion. It reports on the survival, growth performance and gender ratio of triploid and diploid siblings for these four families over a 10 month rearing period.

2. Materials and methods

2.1. Broodstock, spawning and embryo collection

Six month old *P. japonicus* broodstock were obtained from a commercial farm in south-east Queensland, Australia. Broodstock were maintained (25 females: 20 males) in 2000-l round fibreglass tanks, each fitted with a sub-sand circulation system (Crocos and Coman, 1997). Tanks received 1.6-l min^{-1} of 10 μm filtered, 34 ppt salinity seawater at 27±2 °C. Photoperiod was reversed and maintained at 12 h light:12 h dark, with light intensity being reduced in each tank by 10 mm bronze tinted twin wall polycarbonate Polygal® lids. Shrimp were fed with commercial *P. japonicus* pellets (Lucky Star, Taiwan Hung Kuo Industrial Co.) *ad libitum* once per day and chopped fresh squid (2 cm^3) (*Loligo* spp.) three times a week during dark hours.

After acclimation to the reversed light cycle for 30 days, gravid (stage IV; Crocos and Kerr, 1983) females were selected by shining a torch beam through their dorsal exoskeleton during dark hours (Crocos and Coman, 1997). Selected females were then checked for impregnation and induced to spawn by unilateral eye-stalk ablation. Females were spawned in 100-l circular tanks that were filled to 40-l and fitted with an automated spawning detection system (Coman et al., 2003). Spawning tanks were maintained on the same photoperiod and water supply described above (at a flow rate of 0.2-l min^{-1}). Females in spawning tanks were fed with one piece of chopped fresh squid (2 cm^3) (*Loligo* spp.) daily during light hours.

The initiation of spawning, as detected by the automated detection system, was taken as time zero. At 5 min post-spawning detection (psd) approximately 2500 embryos were siphoned into each of four 1-l beakers (i.e. 10,000 embryos total). Siphoning was completed by 7 min psd. Once embryos had settled (approx. 30–40 s), water was poured out of the beakers to leave a volume of 200 ml which contained the majority (>80%) of the siphoned embryos.

2.2. Triploid induction and preliminary polyploidy analysis

At 8–10 min psd 200 ml of a 300 μM 6-DMAP stock solution (freshly made up in 27 °C seawater) was added to each of three of the beakers containing 200 ml of seawater and embryos to give a final treatment concentration of 150 μM 6-DMAP. At the same time 200 ml of seawater was added to the fourth beaker which became the control. Embryos were treated for a 10 min duration after which time each beaker was filled with seawater to 1-l, embryos allowed to settle (approx. 30–40 s) and the water was poured off to leave a volume of 200 ml. This rinsing process was repeated four times for each beaker after which time they were equally divided and stocked into two 9-l hatching tanks, giving two replicates for each treatment and the control. Embryos were incubated at 27±2 °C with light aeration.

Triploid induction rate was initially assessed by analysing a sample of 50 pooled nauplii from each treatment and control replicate. In some instances samples were frozen on the day of hatching and stored at −20 °C for up to 10 days (when these samples were analyzed they were transported to the laboratory frozen and defrosted on ice). If ploidy analysis could be completed on the day of hatching, nauplii were sampled and taken live to the laboratory. Live nauplii were chilled on ice at the laboratory until they died. Once dead nauplii had settled, excess seawater was removed, so less than 200 μl of seawater and sample remained in each tube. Samples were then processed and analyzed using the protocol outlined by Norris et al. (2005). In summary, 500 μl of marine phosphate buffered solution (MPBS) (11.0 g l^{-1} NaCl, 0.2 g l^{-1} KCl, 1.15 g l^{-1} $Na_2HPO_4 \cdot 2H_2O$) propidium iodide (PI) stain (MPBS containing 0.1% Triton X-100, 0.2 mg ml^{-1} Rnase A, 0.02 mg ml^{-1} PI) was added to each sample. Samples were then homogenized and 10 μl of a 1:100 dilution of the internal standard, glutaraldehyde fixed, chicken red blood cells (CRBC) was added to each sample (Handbook of Flow Cytometry Methods, 1993). Cell suspensions were screened through 62 μm mesh prior to fluorescent activated cell sorting (FACS) on a Calibur Flow Cytometer (Beckton Dickinson Immunocytometry Systems San Jose, California, USA). The level of polyploidy in each sample was analyzed using ModFit software (Verity Software House, Topsham, Maine, USA). Four families with greater than 70% triploidy were selected for rearing through to 10 months of age.

2.3. Rearing conditions

A total of four families were reared in two consecutive years, with two families being reared each year. Rearing conditions were consistent between years and families. However, refinement of techniques did occur between years one and two, allowing tagging and ploidy tissue sampling to occur at a smaller size, and for every individual in the second year (families 3 and 4). For each year, two families determined to have greater than 70% triploidy from the preliminary ploidy analyses of the nauplii, were reared to 10 months of age (i.e. families 1 and 2 in the first year; families 3 and 4 in the second year).

For each selected family nauplii from both replicate hatching tanks, for all three 6-DMAP treatments and the control group, were reared to PL10 in 100-l static water larval rearing tanks at 100 larvae l^{-1}. Tanks were heated to 27 °C and received a 50% water exchange every second day using the same water source described above. Larvae were fed with a combination of commercially available formulated *P. japonicus* larval feeds and microalgae (*Chaetocerous muelleri*) or *Artemia* sp. nauplii according to their life history stage (Coman, 2002). Tanks received moderate aeration and a photoperiod of 12 h light:12 h dark. Tanks had translucent white lids to reduce light intensity.

Table 1
Number of *Penaeus japonicus* on which FACS analysis was performed and percentage of analyzed shrimp within each ploidy level for each sex in the different control and 6-DMAP treated shrimp categories

Family	Control		6-DMAP treatment				
	Male	Female	Male		Female		
	Diploid	Diploid	Diploid	Mosaic	Diploid	Mosaic	Triploid
1	100% (*n* = *3*)	100% (*n* = *3*)	0% (*n* = *0*)	100% (*n* = *2*)	0% (*n* = *0*)	7.70% (*n* = *1*)	92.30% (*n* = *12*)
2	100% (*n* = *2*)	100% (*n* = *4*)	0% (*n* = *0*)	100% (*n* = *1*)	0% (*n* = *0*)	9.10% (*n* = *1*)	90.90% (*n* = *10*)
3	100% (*n* = *4*)	100% (*n* = *8*)	94.10% (*n* = *16*)	5.90% (*n* = *1*)	37.50% (*n* = *24*)	0% (*n* = *0*)	62.50% (*n* = *40*)
4	100% (*n* = *3*)	100% (*n* = *5*)	100% (*n* = *43*)	0% (*n* = *0*)	66.10% (*n* = *37*)	1.80% (*n* = *1*)	32.10% (*n* = *18*)

Analysis conducted at PL251 for families 1 and 2 and at PL173 for families 3 and 4. Sample size is italicised and in parentheses below percentage values.

Once larvae reached PL10 (10 days after metamorphosis from mysis to postlarval stage 1) they were transferred into round 100-l plastic, flow through tanks (59 cm dia., 35 cm water depth) at stocking densities of no greater than 2 postlarvae l^{-1}. Tanks received continual aeration and 0.6-l min^{-1} of the seawater source described above. Photoperiod and tank lids were the same as for the previous rearing phase. Larvae were fed with commercially available *P. japonicus* formulated diets and *Artemia* sp. nauplii twice daily.

At PL47 (families 1 and 2) or PL61 (families 3 and 4), up to 150 shrimp were stocked into 2000-l tanks identical to those used for maintaining broodstock. Each replicate of each family was stocked into a separate tank. Water supply was the same as for the broodstock tanks, however, photoperiod was not reversed but still remained at 12 h light:12 h dark. Shrimp were fed with commercially available *P. japonicus* pellets daily which increased in size according to shrimp size. Frozen squid (*Loligo* spp.), cut to 0.2–2 cm^3 pieces depending on shrimp size was fed every second day.

Once shrimp reached PL187 for families 1 and 2 and PL118 for families 3 and 4, they were individually tagged using internal elastomer implants (Northwest Marine Technology Inc.) for family identification and uniquely numbered silicone eye-stalk tags (manufactured on site) for individual identification. After tagging, treatment and control shrimp were mixed to eliminate tank effects on their performance by randomly allocating shrimp within each family across 6 or 8 rearing tanks. From tagging size through the completion of the growout, densities were maintained at <100 shrimp m^{-2}.

2.4. Ploidy analysis of individual shrimp

The ploidy status of individual animals within the four families was assessed at an age after which shrimp could be uniquely eye-stalk tagged, and when non-destructive pleopod and haemolymph samples could be taken. This was completed at PL251 for families 1 and 2, with a sub-sample of control and treatment shrimp being randomly selected for FACS analysis (Table 1). For families 3 and 4 the ploidy status was determined for every individual, in each replicate of the treatment groups and for a random sub-sample of control shrimp. The refinement of the techniques allowed these samples to be collected much earlier, at PL118, in the second growout year (Table 1).

Table 2
Percentage of females for control and 6-DMAP treated *Penaeus japonicus* at different life history stages once gender had been determined

	PL118	PL173	PL227	PL251	PL262	PL284	PL306
Family 1							
Control				48 (*n* = *87*)	52 (*n* = *33*)		
6-DMAP treatment				88 (*n* = *41*)*	88 (*n* = *17*)*		
Family 2							
Control				46 (*n* = *76*)	47 (*n* = *34*)		
6-DMAP treatment				96 (*n* = *45*)*	92 (*n* = *12*)*		
Family 3							
Control	59 (*n* = *126*)	55 (*n* = *96*)	46 (*n* = *52*)			46 (*n* = *61*)	54 (*n* = *37*)
6-DMAP treatment	79 (*n* = *118*)*	77 (*n* = *105*)*	78 (*n* = *72*)*			75 (*n* = *59*)*	70 (*n* = *27*)
Family 4							
Control	51 (*n* = *41*)	46 (*n* = *35*)	nd			nd	nd
6-DMAP treatment	57 (*n* = *146*)	54 (*n* = *118*)	54 (*n* = *92*)			57 (*n* = *83*)	54 (*n* = *57*)

nd not determined.
Sample size is italicised and in parentheses below percentage values.
*Indicates significant deviation from a 50:50 gender ratio as determined by χ^2 test (F > 3.84, P < 0.05).

Pleopod and haemocyte samples were taken simultaneously from individual shrimp, with the pleopod only being used for FACS analysis when insufficient numbers of haemocytes were collected. From each individual one pleopod was cut with sharp, sterile scissors at its base where it joined the abdomen. This was immediately placed in a sterile tube and stored on ice. Once the pleopod was removed, 50–200 μl of haemolymph was collected immediately from the wound using a 200 μl air displacement pipette with a sterile pipette tip that had the end 2 mm cut off to increase the diameter of the aperture. After collection, haemolymph was immediately suspended in 500 μl of chilled shrimp saline solution (SSS) (450 mM NaCl, 10 mM KCl, 10 mM EDTA Na_2, 10 mM HEPES, pH to 7.3) to prevent coagulation, and the sample was stored on ice. Once all shrimp had been sampled, haemocytes were centrifuged at 80 × gravity for 10 min. Supernatant was then removed down to 50–100 μl. Tubes were gently flicked to resuspend the haemocyte pellet and stored on ice.

To each haemocyte sample 500 μl of MPBS PI stain and 10 μl of a 1:100 dilution of CRBC were added. Cell suspensions were screened through 62 μm mesh just prior to FACS as described above. When pleopods were required for FACS analysis they were homogenized using a mini-pestle in 500 μl of MPBS PI stain. After homogenization 10 μl of the 1:100 CRBC dilution was added, and the sample was screened through a 62 μm, mesh just prior to FACS. The ploidy status of each individual was determined using ModFit software (Verity Software House, Topsham, Maine, USA).

2.5. Performance assessments and statistical analysis

To calculate survival performance the number of shrimp within each treatment and control replicate for families 1 and 2 were counted at PL10, PL187 (tagging size) and PL251(assessment age), and for families 3 and 4 at PL10, PL118 (tagging size) and PL173(assessment age). The gender of each shrimp was determined at tagging size for families 3 and 4, but not until assessment age for families 1 and 2; all individuals were weighed from each replicate at the time of gender determination. For families 3 and 4 the ploidy status of every individual was known from PL118. All 6-DMAP treated diploid and triploid females were weighed at PL227, PL284 and PL306 for families 3 and 4. For families 1 and 2 the total number of males and females in the control and 6-DMAP treated groups were determined at PL251 and PL262. For families 3 and 4 data was collected to determine gender ratios at PL118, PL173, PL227, PL284 and PL306.

Survival and growth of control and 6-DMAP exposed shrimp, and growth of diploid and triploid shrimp within the 6-DMAP treated groups, were analyzed

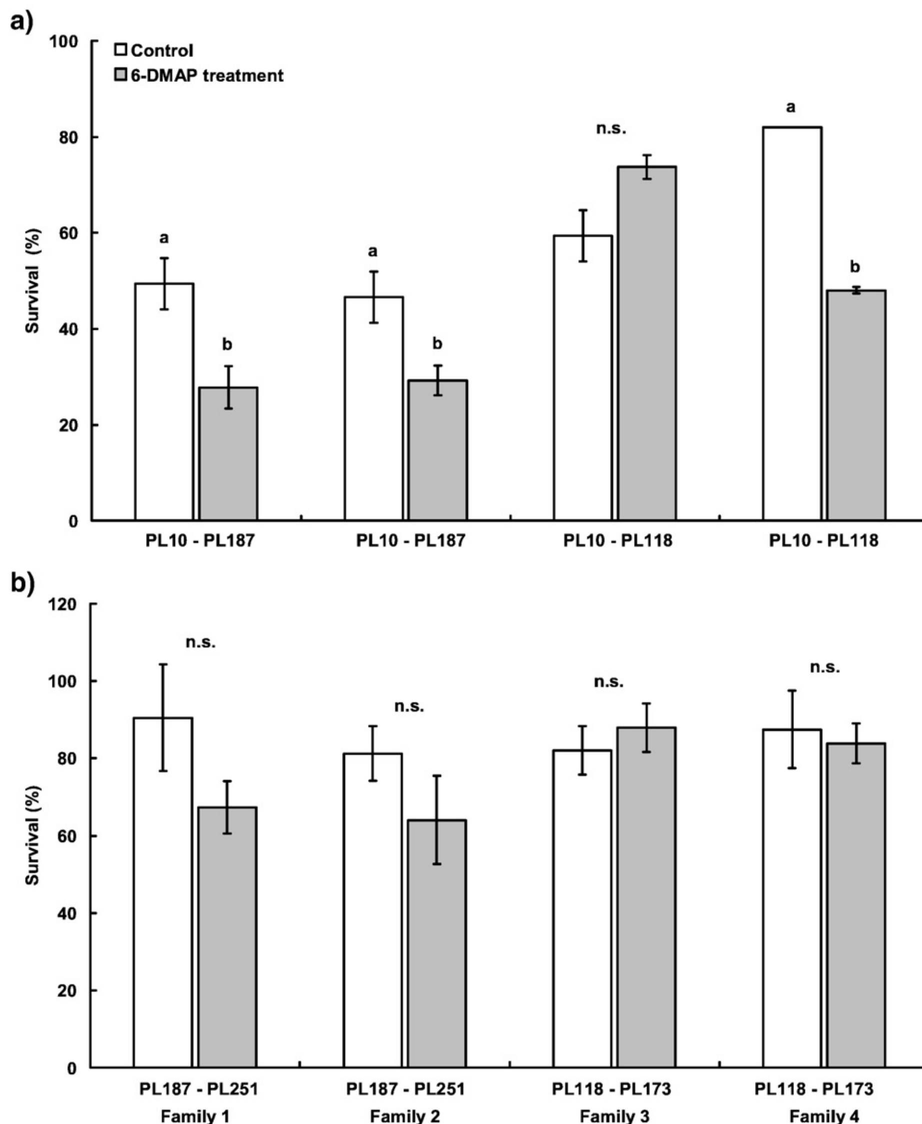

Fig. 1. Percentage survival (±SE) of control and treatment *Penaeus japonicus* for all four families from a) PL10 to tagging size, and b) tagging to assessment age. Each category includes shrimp of both genders, with the treatment category also including shrimp of all three ploidy levels; diploid, mosaic and triploid. Bars with different superscripts were significantly different within family ($P < 0.05$).

separately for each family using analysis of variance (PROC GLM; SAS Institute Inc., 1999). Percentage survival in replicate tanks was used to analyze survival and individual weights were used to assess growth.

3. Results

3.1. Ploidy assessments

From the preliminary FACS analysis of nauplii the average percentage triploidy in the 6-DMAP treatment groups for families 1, 2, 3 and 4 were 73.3±1.75%, 76.5±3.57%, 93.4±2.53%, and 84.3±0.06% respectively. From the individual FACS analysis of haemocytes or pleopods taken at tagging size for families 3 and 4 and at PL251 for families 1 and 2, all control shrimp from all families were diploid as expected (Table 1). No male triploids were recorded; however, there were four mosaic males, which had both triploid and diploid cell populations. Three females were also found to be mosaic. All individuals identified as triploid by FACS analysis were distinguished as phenotypically female with the highest induction rates calculated from the analyzed cohort of individuals from family 1 at 92.3%, followed by families 2, 3 and 4 at 90.9%, 62.5% and 32.1% respectively (Table 1).

3.2. Gender ratio

For all families the ratio of males: females in control groups were not skewed for any of the assessed life history stages (Table 2). In comparison, gender ratios skewed towards females were recorded for all treatment groups of families 1, 2 and 3 at all assessed life history stages (Table 2). The gender ratios for family 4 were marginally skewed towards the females at the different assessment ages (Table 2).

3.3. Effect of 6-DMAP treatment on survival

Survival from PL10 until tagging size (i.e. PL10–PL187) was significantly lower in the 6-DMAP treatment groups than the controls for family 1 and family 2 (Fig. 1a, Table 3). Survival of family 4 from PL10 to tagging size (PL118) was significantly lower in the 6-DMAP treatments than the controls (Table 3), but for family 3 survival was higher for the treatments than the controls however, this difference was not statistically significant, and was influenced by poor survival in a single replicate tank of control animals (Fig. 1a). No significant difference in survival from tagging (PL187 for families 1 and 2, PL118 for families 3 and 4) until assessment age (PL251 for families 1 and 2, PL173 for families 3 and 4) was found between the 6-DMAP treatment and controls in any of the families (Fig. 1b). However, while not significant, average survivals in the 6-DMAP treatments were considerably lower than in the controls for families 1 and 2 (Fig. 1b).

3.4. Effect of 6-DMAP treatment on growth

For families 1, 2 and 3, the weight of the females at assessment age in the 6-DMAP treatments were significantly lower than the females in

Table 3
ANOVA results for comparisons of survival and weight

Variable analyzed	Source	*n*	SE	*F* value	*df*	*P* value	Result
Percentage survival of control v treatment[a] animals between different ages							
Family 1 PL10 – PL187	Treatment	14	12.40	9.80	1, 13	0.0087	C>T
Family 2 PL10 – PL187	Treatment	14	11.47	8.00	1, 13	0.0152	C>T
Family 3 PL10 – PL118	Treatment	6	7.23	5.94	1, 5	0.0715	ns
Family 4 PL10 – PL118	Treatment	4	1.16	642.22	1, 3	0.0016	C>T
Family 1 PL187 – PL251	Treatment	16	30.79	2.27	1, 15	0.1544	ns
Family 2 PL187– PL251	Treatment	16	26.87	1.64	1, 15	0.2215	ns
Family 3 PL118 – PL173	Treatment	16	18.69	0.61	1, 15	0.4468	ns
Family 4 PL118 – PL173	Treatment	16	22.40	0.10	1, 15	0.7511	ns
Average weight of control v treatment[b] animals at assessment age for males and females							
Family 1 at PL251-female	Treatment	79	3.86	43.51	1, 78	0.0001	C>T
Family 1 at PL251-male	Treatment	51	2.03	28.28	1, 50	<0.0001	C>T
Family 2 at PL251-female	Treatment	76	5.67	13.92	1, 75	0.0004	C>T
Family 2 at PL251-male	Treatment	49	2.69	0.37	1, 48	0.5440	ns
Family 3 at PL173-female	Treatment	132	3.12	14.45	1, 131	0.0002	C>T
Family 3 at PL173-male	Treatment	64	2.10	0.83	1, 63	0.3665	ns
Family 4 at PL173-female	Treatment	78	2.55	0.10	1, 77	0.7542	ns
Family 4 at PL173-male	Treatment	70	2.01	5.19	1, 69	0.0259	T>C
Average weight of treatment diploid females v treatment triploid females at different ages							
Family 3 at PL173	Ploidy level	60	2.92	4.62	1, 59	0.0358	D>Tr
Family 3 at PL227	Ploidy level	48	4.66	0.31	1, 47	0.5816	ns
Family 3 at PL284	Ploidy level	35	5.08	1.12	1, 34	0.2983	ns
Family 3 at PL306	Ploidy level	18	4.90	0.03	1, 17	0.8542	ns
Family 4 at PL173	Ploidy level	55	2.36	12.72	1, 54	0.0008	D>Tr
Family 4 at PL227	Ploidy level	43	4.12	10.09	1, 42	0.0028	D>Tr
Family 4 at PL284	Ploidy level	35	4.83	5.97	1, 34	0.0201	D>Tr
Family 4 at PL306	Ploidy level	29	4.61	9.31	1, 28	0.0051	D>Tr

C = control, T = treatment, D = diploid, Tr = triploid, ns no significant difference.

[a] Each category includes both genders, with the treatment category also including diploids, triploids and mosaics. All control animals are diploid.

[b] Treatment category includes diploid and triploid females.

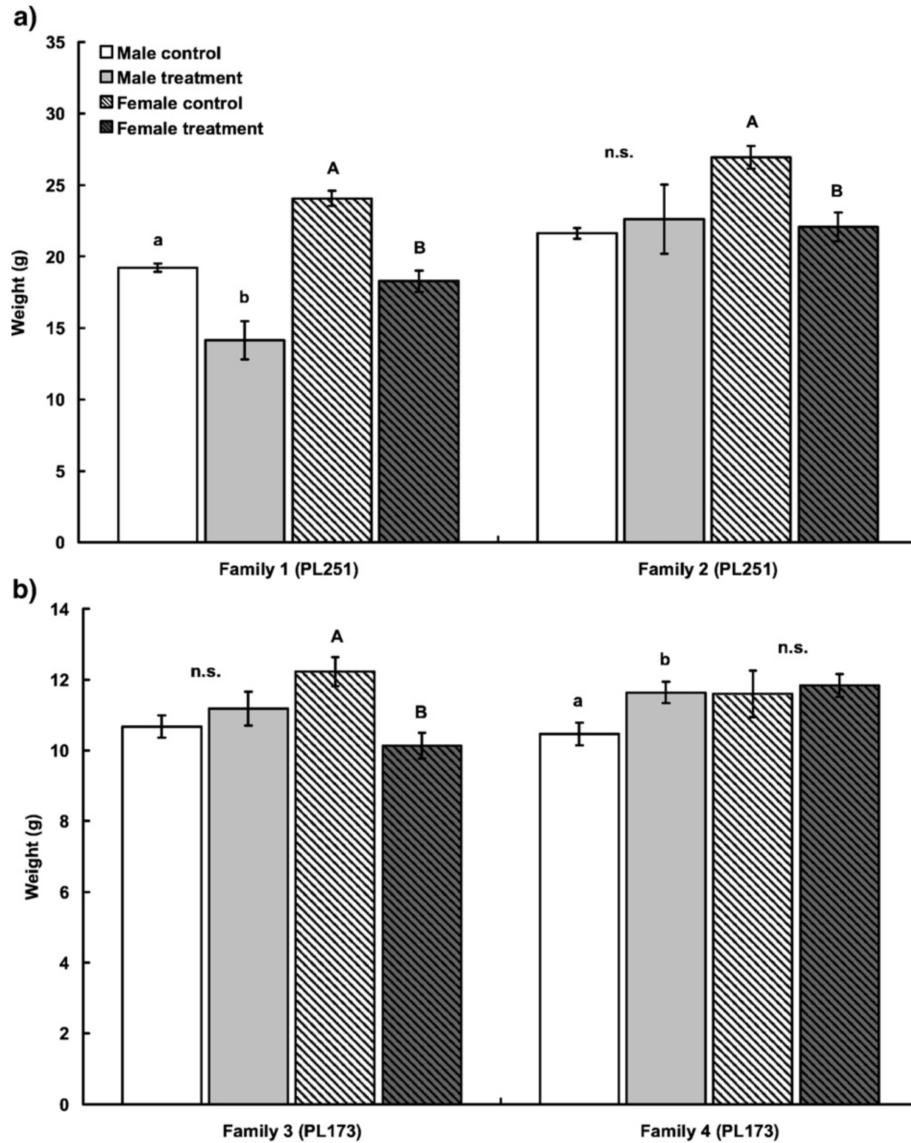

Fig. 2. Average weight (±SE) at assessment age of control and treatment *Penaeus japonicus* for each gender for a) families 1 and 2 at PL251 and b) families 3 and 4 at PL173. Each treatment category includes shrimp of all three ploidy levels; diploid, mosaic and triploid. Bars with different superscripts were significantly different within each gender (males in lower case, females in uppercase) of each family ($P<0.05$).

the control groups (Table 3, Fig. 2). Similarly, the weight of the males from family 1 was significantly lower in the 6-DMAP treatment group than the control group. No difference in assessment age weight was found between control and 6-DMAP treatments for males from families 2 and 3, and females from family 4. Notably, males from family 4 had higher weights at assessment age in the 6-DMAP treatment group than the control group (Fig. 2). Apart from family 4 the size of the control males was equal to or slightly (but not significantly) greater than the treatment females.

3.5. Effect of ploidy level on female growth

Triploid females from both families 3 and 4 weighed significantly less (Table 3) at PL173 than diploid female siblings who had also been exposed to the same 6-DMAP treatment (Fig. 3a, b). However, no differences in weights of triploids and diploids were found at all subsequent measures for family 3, whereas the triploid females from family 4 were significantly smaller than their diploid siblings (Table 3) at all subsequent measures (Fig. 3a, b). As mentioned in the previous section, males were close to equal the size of treatment females, this was due to the triploid females being smaller than the males, while the diploid treatment females were larger than the males, as would normally be expected for penaeids.

4. Discussion

All triploid *P. japonicus* individuals in this study were female resulting in skewed gender ratios in 6-DMAP treated populations. Skewed gender ratios of 1 male:4 females have been previously reported for triploid *P. chinensis* (Li et al., 2003b). However, to our knowledge the fact that all triploids are female seems to be unique to *P. japonicus*. Whilst the skewed gender ratios observed for triploid families could be

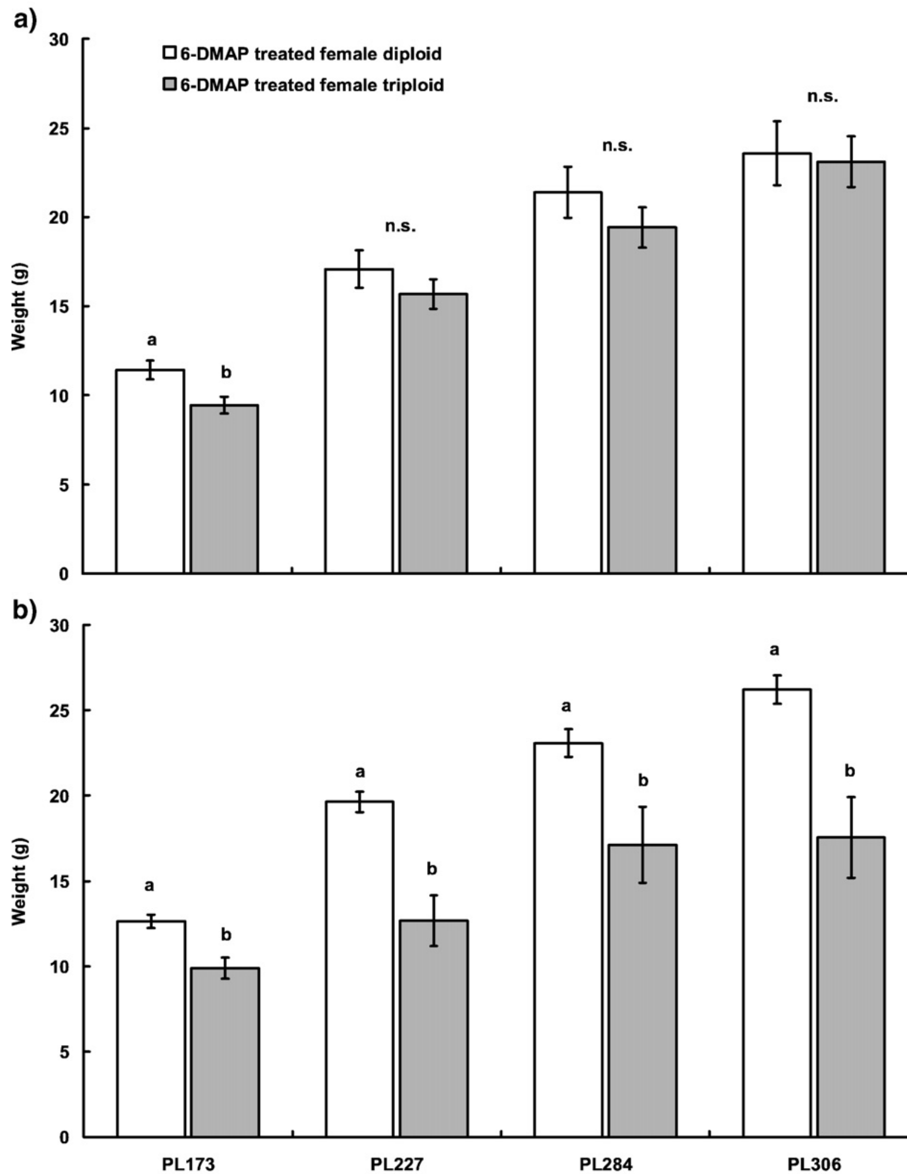

Fig. 3. Average weight (±SE) from assessment age (PL173) to reproductive age (PL306) of treatment diploid and treatment triploid females for a) family 3 and b) family 4. Bars with different superscripts were significantly different within each life history stage ($P<0.05$).

explained by several sex determination pathways previously described for fish species (Van Enennaam et al., 1999; Devlin and Nagahama, 2002), from this study it is not possible to determine which of these pathways penaeid shrimp utilize. Evidence from this and other studies indicates that penaeid shrimp utilizes the ZW–ZZ sex determination. When sex is determined this way females are the heterogametic gender and would therefore be represented as ZW and males as ZZ. Oocytes that have retained polar body II (as in the present study) will either be ZZ or WW, and subsequent fertilization with haploid sperm (Z) will result in triploid progeny that are either ZZZ or WWZ. If the female chromosome is overdominant (i.e. WWZ offspring are phenotypic but sterile females) and the ZZZ genotype is not viable, the gender ratio of the offspring from triploid inductions would be dominated by females and all surviving triploids would be female due to the non-viability of male triploids. Observations from the present study support this hypothesis of female heterogeneity in gender determination of *P. japonicus*. The hypothesis was previously put forward by Benzie et al. (2001) who reported skewed gender ratios dominated by males for hybrid crosses of *Penaeus monodon* and *P. esculentus*, and concluded that penaeid females may be the heterogametic gender due to the Haldane effect, where the absence of one gender in hybrid progeny suggests that that gender is likely to be heterogametic (Haldane, 1922). Furthermore, Li et al. (2005) have documented that gender-linked markers appear only on the maternal genomic map for *P. japonicus* supporting the hypothesis that female *P. japonicus* are heterogametic.

Some 6-DMAP treated male and female *P. japonicus* from this study were found to be mosaic (having diploid and triploid cell populations). This phenomenon has been documented previously when using shock agents to prevent early embryonic processes in crustaceans (Qiu et al., 1997; Xiang et al., 1992) and is thought to be the result of incomplete inhibition of mitosis in cells that have two or more nuclei. As with the current study, Xiang et al. (1992) found only a low occurrence of mosaics, but Qiu et al. (1997) found greater than 18% of embryos induced by heat shock were mosaic. Bidwell et al. (1985), working with channel catfish (*Ictalurus punctatus*), had cases of 100% mosaics under some induction conditions. To our knowledge there are no reports on growth performance of mosaics in comparison to diploids, which is likely to be due to the low number of mosaics that are normally encountered. Without greater knowledge about the growth potential of mosaics they are not likely to play a major role in commercial production.

The percentage of triploids within a treatment population decreased noticeably for families 3 and 4 from nauplius stage to when they reached tagging size (PL118) potentially indicating that juvenile triploids were less viable than diploids. However, from the sub-adult age onwards (i.e. PL118), survival performance of triploid females is similar to that of diploid female siblings (no data on ploidy status of families 1 and 2 was available until PL251). This suggests that beyond a certain size, triploidy has no detrimental effect on survival. This result has also been observed for other triploid families reared at CSIRO Marine and Atmospheric Research (Unpublished data). Although survival data for this study cannot conclusively confirm this hypothesis (because it is not possible to take a non-destructive cell sample from individuals for FACS until shrimp are more than 5 g in size), such observations have been made elsewhere for bivalve (Downing, 1988; Nell et al., 1995; Wang et al., 1998; Kudo et al., 2000) and fish species (Sugama et al., 1992; Fast et al., 1995; O'Flynn et al., 1997).

In some instances treatment with 6-DMAP had a negative impact on growth up until adult size for male and female *P. japonicus*. When used to produce triploid bivalves it has been found that 6-DMAP can negatively impact growth, survival and development particularly in the early stages of development (Desrosiers et al., 1993; Scarpa et al., 1995). It is possible that the toxicity of 6-DMAP slows early embryonic development and larval growth of shrimp, resulting in treated shrimp which are always going to be smaller than their untreated siblings.

Triploid female shrimp were generally found to be smaller than 6-DMAP treated diploid females in this study. Despite improved growth rates of triploids over diploids being a well documented phenomenon, particularly for bivalves (Beaumont and Fairbrother, 1991; Benfey, 1999; Brake et al., 2004), there are also reports of triploids being inferior in growth performance to their diploid counterparts (Benfey and Sutterlin, 1984; Lincoln and Scott, 1984; Henken et al., 1987). Generally the improved growth of triploid animals is thought to be linked to their inability to reproduce because they are unable to produce normal gametes, making more energy available for somatic growth. Xiang et al. (2006) obtained results supporting this when immature triploid *F. chinensis* did not out perform their diploid siblings until after the diploids had reached sexual maturity. However, in this current study, the detrimental effect on growth rates was noticeable well before sexual maturity which meant that by the time diploid female animals had diverted energy to reproduction, rather than growth, they would already be significantly bigger than their triploid female siblings.

The production of all female shrimp families would still generally be expected to result in better overall growout production, as mature diploid female penaeids are larger than males. However, the average weight of the treatment females was at best equal to, but was usually smaller than the diploid males. Furthermore, triploid females from the treatments were smaller than the treated diploid females. Production from a pond stocked with triploid *P. japonicus*, produced as described in this study, would therefore be far smaller than production from a pond stocked with male and female diploid animals produced using regular hatchery methods. At present the production of all female populations would not appear to have any production advantages, but may still be useful to reduce the possibility of genetically altered shrimp breeding if they were to escape into nearby natural waterways or to prevent unauthorised breeding from selectively improved stocks that have been exported live. Induction techniques that have no detrimental impact on triploid growth and survival performance but still result in high numbers triploid offspring may provide an economic benefit to commercial production, and should be further investigated. Techniques for inducing triploidy that have been successful for some other species include temperature and pressure shocks (Bidwell et al., 1985; Shen et al., 1993; Li et al., 1999; Kozfkay et al., 2005).

In conclusion, the discovery that all triploid *P. japonicus* are female could be of significant commercial interest in the future. However, triploidy induced by 6-DMAP negatively impacts growth and survival performance from sub-adult to reproductive size when compared to diploid siblings. Future studies would benefit from investigating alternative induction strategies and protocols in an effort to improve triploid growth and survival performance. Studies on the reproductive capacity of *P. japonicus* triploids are also imperative and are currently underway at CSIRO Marine and Atmospheric Research.

Acknowledgements

The authors would like to thank Stuart Arnold for his experimental support, and Paula Hall and Grace Chojnowsky from the Queensland Institute of Medical Research for their assistance with flow cytometry. We would also like to acknowledge Dean Jerry, Sandy Keys and Simon Tabrett for comments on drafts of the manuscript.

References

Bao, Z., Zhang, Q., Wang, H., 1994. Cytochalasin B induced triploidy in *Penaeus chinensis*. Acta Oceanologica Sinica 13 (2), 261–267.

Beaumont, A.R., Fairbrother, J.E., 1991. Polyploidy manipulation in molluscan shellfish: a review. Journal of Shellfish Research 10 (1), 1–17.

Benfey, T.J., 1999. The physiology and behaviour of triploid fishes. Reviews in Fisheries Science 7 (1), 39–67.

Benfey, T.J., Sutterlin, A.M., 1984. Growth and gonadal development of triploid landlocked Atlantic salmon (*Salmo salar)*. Canadian Journal of Fisheries and Aquatic Sciences 41, 1387–1392.

Benzie, J.A.H., Kenway, M., Ballment, E., 2001. Growth of *Penaeus monodon* X *Penaeus esculentus* tiger shrimp hybrids relative to parental species. Aquaculture 193, 227–237.

Bidwell, C.A., Chrisman, C.L., Libey, G.S., 1985. Polyploidy induced by heat shock in channel catfish. Aquaculture 51, 25–32.

Brake, J., Davidson, J., Davis, J., 2004. Field observations on growth, gametogenesis, and sex ratio of triploid and diploid *Mytilus edulis*. Aquaculture 236, 179–191.

Coman, G.J., 2002. Factors affecting the efficiency of genetic selection of the Kuruma prawn, *Penaeus japonicus.* Thesis for Doctor of Philosophy, Department of Zoology and Entomology, The University of Queensland, Australia. p. 21–22.

Coman, F., Norris, B., Pendrey, R., Preston, N., 2003. A simple spawning detection and alarm system for penaeid shrimp. Aquaculture Research 34, 1359–1360.

Crocos, P.J., Coman, G.J., 1997. Seasonal and age variability in the reproductive performance of *Penaeus semisulcatus* broodstock: optimising broodstock selection. Aquaculture 155, 55–67.

Crocos, P.J., Kerr, J.D., 1983. Factors affecting induction of maturation and spawning of the tiger prawn *Penaeus esculentus* (Haswell), under laboratory conditions. Aquaculture 58, 203–214.

Desrosiers, R.R., Gerard, A., Peignon, J.-M., Naciri, Y., Dufresne, L., Morrasse, J., Ledu, C., Phelipot, P., Guerrier, P., Dube, F., 1993. A novel method to produce triploids in bivalve molluscs by the use of 6-dimethylaminopurine. Journal of Experimental Marine Biology and Ecology 170, 29–43.

Devlin, R.H., Nagahama, Y., 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208, 191–364.

Downing, S.L., 1988. Optimal induction of triploidy in *Crassostrea gigas* depends on temperature. Journal of Shellfish Research 7, 115 (summary only).

Dumas, S., Ramos, R., 1999. Triploidy induction in the Pacific white shrimp *Litopenaeus vannamei* (Boone). Aquaculture Research 30, 621–624.

Fast, A.W., Pewnim, T., Keawtabtim, R., Saljit, R., Te, F.T., Vejaratpimoi, R., 1995. Comparative growth of diploid and triploid Asian catfish *Clarias macrocephalus* in Thailand. Journal of the World Aquaculture Society 26, 390–395.

Guo, X., Allen Jr., S.K., 1994. Viable tetraploids in the Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body 1 in eggs from triploids. Molecular Marine Biology and Biotechnology 3, 42–50.

Haldane, J.B.S., 1922. Sex ratio and unisexual sterility of hybrid animals. Journal of Genetics 12, 101–109.

Hand, R.S., Nell, J.A., Thompson, P.A., 2004. Studies on triploid oysters in Australia. XIII. Performance of diploid and triploid Sydney rock oyster, *Saccostrea glomerate* (Gloud 1850), progeny from a third generation breeding line. Aquaculture 233, 93–107.

Heasman, M.P., O'Connor, W.A., O'Conner, S.J., Walker, S., 1998. Enhancement and farming of scallops in NSW using hatchery produced seedstock, volume 1. NSW Fisheries Final Report Series, Cronulla NSW, 8. 146 pp.

Henken, A.M., Brunink, A.M., Richter, C.J.J., 1987. Differences in growth rate and feed utilization between diploid and triploid African catfish, *Clarias gariepinus* (Burchell 1822). Aquaculture 63, 233–242.

Hewitt, D., Duncan, P., 2001. Effect of high water temperature on the survival, moulting and food consumption of *Penaeus* (*Marsupenaeus*) *japonicus* (Bate, 1888). Aquaculture Research 32, 305–311.

Kozfkay, J., Wagner, E., Aplanalp, D., 2005. Production of triploid lake trout by means of pressure treatment. North American Journal of Aquaculture 67, 93–97.

Kudo, M., Arai, K., Kimoto, T., Minagawa, M., Fujino, K., 2000. Survival, growth, and maturation of triploid abalone, *Haliotus diversicolor diversicolor*. Report of the Tokyo Metropolitan Fisheries Experiment Station 212, 170–179.

Li, F., Zhou, L., Xiang, J., Liu, X., Zhu, J., 1999. Triploidy induction with heat shocks to *Penaeus chinensis* and their effects on gonad development. Chinese Journal of Oceanology and Limnology 17, 57–61.

Li, F., Xiang, J., Zhou, L., Wu, C., Zhang, X., 2003a. Optimization of triploid induction by heat shock in Chinese shrimp *Fenneropenaeus chinensis*. Aquaculture 219, 221–231.

Li, F., Xiang, J., Zhang, X., Zhou, L., Zhang, C., Wu, C., 2003b. Gonad development characteristics and sex ratio in triploid Chinese shrimp (*Fenneropenaeus chinensis*). Marine Biotechnology 5, 528–535.

Li, Y., Byrne, K., Miggiano, E., Whan, V., Moore, S., Keys, S., Crocos, P., Preston, N., Lehnert, S., 2005. Genetic mapping of the kuruma prawn *Penaeus japonicus* using AFLP markers. Aquaculture 219, 143–156.

Lin, F., Cai, N., 1996. Triploid induction in *Penaeus chinensis*. Studia Marina Sinica/Haiyang Kexue Jikan 37, 79–89.

Lincoln, R.F., Scott, A.P., 1984. Sexual maturation in triploid rainbow trout *Salmo gairdneri* Richardson. Journal of Fish Biology 25, 385–392.

Nam, Y.K., Park, I., Kim, D.S., 2004. Triploid hybridization of fast-growing transgenic mud loach (*Misgurnus mizolepis*) male to cyprinid loach (*Misgurmus anguillicaudatus*) female: The first performance study on growth and reproduction of transgenic polyploid hybrid fish. Aquaculture 231, 559–572.

Nell, J., 2002. Farming triploid oysters. Aquaculture 210, 69–88.

Nell, J.A., O'Connor, W.A., Hand, R.E., McAdam, S.P., 1995. Hatchery production of diploid and triploid clams, *Tapes dorsatus* (Lamarck 1818): a potential new species for aquaculture. Aquaculture 130, 389–394.

Norris, B.J., Coman, F.E., Sellars, M.J., Preston, N.P., 2005. Triploid induction in *Penaeus japonicus* (Bate) with 6-dimethylaminopurine. Aquaculture Research 36, 202–206.

O'Flynn, F., McGeachy, S.A., Friars, G.W., Benfey, T.J., Bailey, J.K., 1997. Comparisons of cultured triploid and diploid Atlantic salmon (*Salmo salar* L.). ICES Journal of Marine Science 54, 1160–1165.

Peyre, J.F.L., Cooper, R.K., Supan, J.E., Volety, A.K., 1999. Total bacterial and *Vibrio vulnificus* load in diploid and triploid eastern oysters in Louisiana. Journal of Shellfish Research 18, 324.

Qiu, G.F., Du, N.S., Lai, W., 1997. A preliminary study on induction of tetraploidy in the freshwater prawn *Macrobrachium nipponese* by heat shock. Journal of Fisheries of China 21, 13–17.

Robinson, J.P. (Ed.), 1993. Handbook of Flow Cytometry Methods. Wiley-Liss, New York.

SAS Institute Software, 1999. SAS/STAT Software, Version 7. SAS Institute Inc., Cary, NC, USA.

Scarpa, J., Vaughan, D.E., Longley, R., 1995. Induction of triploidy in the eastern oyster, *Crassostrea virginica*, using 6-DMAP. Journal of Shellfish Research 14 (summary only).

Sellars, M.J., Degnan, B.M., Preston, N.P., 2006. Induction of triploid Kuruma shrimp, (*Marsupenaeus (Penaeus) japonicus* (Bate) nauplii through inhibition of polar body I, or polar body I and II extrusion using 6-dimethylaminopurine. Aquaculture 256, 337–345.

Shen, Y., Zhang, X.-Y., He, H.-P., Ma, L.-J., 1993. Triploidy induction by hydrostatic pressure in the pearl oyster, *Pinctada martensii* Dunker. Aquaculture 110, 221–227.

Stanley, J.G., Allen, S.K., Hidu, H., 1981. Polyploidy induced in the early embryo of the American oyster with cytochalasin B. Journal of Shellfish Research 1, 123.

Stepto, N.K., Cook, P.A., 1998. Induction of triploidy in the South African abalone using cytochalasin B. Aquaculture International 6, 161–169.

Sugama, K., Taniguchi, N., Seki, S., Nabeshima, H., 1992. Survival, growth and gonad development of triploid red sea bream, *Pagrus major* (Temminck et Schlegel): use of allozyme markers for ploidy and family identification. Aquaculture and Fisheries Management 23, 149–159.

Tave, D., 1993. Genetics for Fish Hatchery Managers, Second edition. Van Nostrand Reinhold, New York. 415 pp.

Van Enennaam, A.L., Van Enennaam, J.P., Medrano, J.F., Doroshov, S.I., 1999. Evidence of female heterogametic sex determination in white sturgeon. Journal of Heredity 90, 231–233.

Vadopalas, B., Davis, J., 1998. Induction of triploidy in the geoduck clam, *Panope abruta*. Journal of Shellfish Research 17, 11–18.

Wang, Z., Wang, R., Yu, R., Tian, C., 1998. Biological characteristics of polyploid shellfish. Journal of the Ocean University of Qingdao/Qingdao Haiyang Daxue Xuebao 28, 399–404 (Qingdao).

Xiang, J.H., Zhou, L.H., Liu, R.Y., Zhu, J.Z., Li, F.H., Li, X.H., Liu, X.D., 1992. Induction of the tetraploids of the Chinese shrimp *Penaeus chinensis*. Biotechnology in Agriculture, Proceedings of the first Asia-Pacific Conference, Beijing, pp. 496–501.

Xiang, J., Li, F., Zhang, X., Yu, K., Zhou, L., Wu, C., 2006. Evaluation of induced triploid shrimp *Penaeus* (*Fenneropenaeus*) *chinensis* cultured under laboratory conditions. Aquaculture 259, 108–115.

Zhang, G., Wang, Z., Chang, Y., Song, J., Ding, J., Wang, Y., Wang, R., 1998. Triploid induction in Pacific abalone *Haliotis discus hannai* Ino by 6-dimethylaminopurine and the performance of triploid juveniles. Journal of Shellfish Research 17, 783–788.

Zhang, X., Li, F., Xiang, J., 2003. Chromosome behaviour of heat shock induced triploid in *Fenneropenaeus chinensis*. Chinese Journal of Oceanology and Limnology 21, 222–228.

Zhang, C., Li, F., Yu, K., Wu, C., Guo, Z., Xiang, J., 2004. Haematological changes of sibling diploid and triploid *Fenneropenaeus chinensis* after being challenged with pathogens. Journal of Fisheries of China/Shuichan Xuebao 28, 535–540.