

Potential application of triploidy induction in important aquatic species in South East Asia

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ABSTRACT

Triploidy, a condition of having 3 sets of chromosomes in the nucleus, occurs naturally in fish. Induction of triploidy is possible by subjecting newly fertilized eggs to sub-lethal treatments prior to the extrusion of polar bodies. The purposes of the induction include improving growth and/or obtaining sterile animals for various purposes. This article gives a brief background of triploid induction in aquatic animals, performances of triploids, collective information on triploidy induction in aquatic species native to South East Asia, and future directions.

INTRODUCTION

Polyploidy is relatively common in fish compared to higher vertebrates. Many fish taxa, namely, Salmonids, Cobitids, Silurids, etc. are known to have evolved through polyploidization of the genome (reviewed by Leggatt and Iwama, 2003). Furthermore, among diploid species, spontaneous polyploidy fishes were reported in at least 20 species, e.g., *Misgurnus anguillicaudatus*, *Carassius carassius gibelio*, *Oncorhynchus mykiss*, etc. (reviewed by Leggatt and Iwama, 2003). Most of polyploidfish found in nature are triploid which presumably arises from the fertilization of a diploid egg with a normal sperm. This knowledge has paved the way to artificial induction of triploidy in fish, shellfish and crustaceans.

Triploids have been induced since early the 1970s in many species of fish (Swarup, 1959; Purdom, 1972; Valenti, 1975; Refstie *et al.*, 1977), shellfish (Stanley *et al.*, 1984; Jiang *et al.*, 1993) and crustaceans (Parsons, 1993) aiming at different advantages over the diploid counterpart. These include improving growth rate due to the additional genome or due to sterility, avoidance of deteriorated meat quality after maturation, and production of sterile animals to avoid genetic contamination or overpopulation.

Induction of triploidy can be achieved by applying sub-lethal treatments to newly fertilized eggs. The sub-lethal treatment could be high temperature (heat shock) or cold shock. In general, cold shock is more effective when applied to tropical species while heat shock is suitable for cold species with some exceptions (Linhart *et al.* 1991; Tiwary *et al.* 2004; Dias da Silva *et al.* 2007). Hydrostatic pressure shock is more effective than the temperature shock but it cannot accommodate large amount of eggs (Thorgaard, 1986; Hussain *et al.*, 1991). Chemical shock (e.g. Cytochalasin B, Ruiz-Verdugo *et al.*, 2001; 6-dimethylaminopurine, Sellars *et al.*, 2012a, b) is also effective and has frequently been applied in shellfish and crustaceans.

To optimize the induction protocol the following parameters are important. Timing at commencement of the induction should be soon

after water activation of sperm before the extrusion of first- (in case of shellfish) or second polar body. The duration of the induction should be long enough so that the retention of the polar body is completed (Felip *et al.* 1997). These factors affect both the success rate of obtaining triploidy and also the hatching rates of the shocked embryos. Some of the protocols for induction of triploidy aquatic animals

were shown in Table 1.

The verification of triploids has mainly depended on chromosome count (Thorgaard, 1983; Nanda *et al.*, 1995), erythrocyte volume measurement (Koedprang and Na-Nakorn, 2000), flow-cytometry (Ruiz-Verdugo *et al.*, 2001; Sellars *et al.*, 2013), or molecular methods, e.g. microsatellite typing (Kang *et al.*, 2013).

Table 1 Induction of triploidy in fishes, shellfishes and crustaceans using different shock means and conditions (Note: psi=pounds/square inches).

Species	Means of shock	Time at commencement	Shock duration	Reference
Fish				
Tilapia, <i>Oreochromis niloticus</i>	Heat shock: 41°C	5 min after fertilization	3.5 min	Hussain <i>et al.</i> (1991)
	Hydrostatic pressure shock: 8000 psi	9 min after fertilization	2 min	
	Cold shock: 9°C	7 min after fertilization	30 min	Hussain <i>et al.</i> (1991)
Günther's walking catfish, <i>Clarias macrocephalus</i>	Cold shock: 7°C	immediately after insemination	25 min	Na-Nakorn and Lakhaanantakun (1993)
	Cold shock: 4°C	2 min after fertilization	15 min	Fast <i>et al.</i> (1995)
African catfish, <i>Clarias gariepinus</i>	Cold shock: 5°C	3 min after fertilization	40 min	Henken <i>et al.</i> (1987)
Common carp, <i>Cyprinus carpio</i>	Heat shock: 40°C	1–3 min after fertilization	1.5 min	Basavaraju <i>et al.</i> (2002)
Atlantic salmon, <i>Salmo salar</i>	Hydrostatic pressure shock: 9500 psi	30 min after fertilization	5 min	Benfey and Sutterlin, (1984)
	Heat shock at 32°C	20 min after fertilization	5 min	Benfey and Sutterlin, (1984)
Rainbow trout, <i>Oncorhynchus mykiss</i>	Cold shock: 6-8°C	15 min after fertilization	15 min	Diaz <i>et al.</i> (1993)

Table 1 (continued)

Species	Means of shock	Time at commencement	Shock duration	Reference
Crustaceans				
Black tiger shrimp, <i>Penaeus monodon</i>	Cold shock: 8°C	8 min post spawning (fertilization)	10 min	Pongtippatee <i>et al.</i> (2012)
	Chemical shock: 200 µM of 6-dimethylaminopurine (DMTP)	6 min 40 sec after spawning (fertilization)	10 min	Sellars <i>et al.</i> (2012a)
Chinese shrimp, <i>Fenneropenaeus chinensis</i>	Heat shock: 29 – 32°C	18 – 20 min after fertilization	10 min	Li <i>et al.</i> (2003)
White shrimp <i>Litopenaeus vannamei</i>	Chemical shock: 200 µm 6-DMAP for duration	1 min post-spawning (fertilization)	8 min	Sellars <i>et al.</i> (2012b)
	Cold shock: 10°C	20 min after fertilization	15 min	Garnica-Rivera <i>et al.</i> (2004)
Shellfish				
Common mussel, <i>Mytilus edulis</i>	Chemical shock: 400 µM of 6-DMAP	21 min after fertilization	20 min	Brake <i>et al.</i> (2002)
Sydney rock oyster, <i>Saccostrea Commercialis</i>	Chemical shock: 0.5 mg/l Cytochalasin B	23 min after fertilization	20 min	Nell <i>et al.</i> (1994)
Pacific oyster, <i>Crassostrea gigas</i>	Chemical shock: 1 mg/l Cytochalasin B at 20 °C	newly fertilized eggs	30-45 min	Allen Jr. and Downing (1986)

Performances of triploids

Growth rate

Triploid fish might be expected to exhibit a higher growth rate than their diploid counterparts due to an additional genome. However, empirical data has shown that growth performances of triploids relative to diploids varied among (reviewed by

Tiway *et al.*, 2004) and within species (e.g. Thai walking catfish, *Clarias macrocephalus*, Na-Nakorn and Lakaanantakun, 1993; Fast *et al.*, 1995). Such the variation may be accounted by strain effects (Taniguchi *et al.*, 1986; Sacobie *et al.*, 2012) or differences of rearing conditions (Flajshans *et al.*, 1993; Benfey, 2001; Flajshans *et al.*, 2004; Taylor *et*

al., 2014). Recently, the study on gene expression revealed that triploid fish sometimes possess a mechanism that silences the expression of alleles from an additional genome. The pattern of allelic expression varied among genes and within a gene in different tissues (Pala *et al.*, 2008; Garcia *et al.*, 2014). This result may partly explain the variation of the growth performance.

Sterility

In general, triploids are functionally sterile due to the irregular meiotic division resulting in aneuploid gametes (Tiwary *et al.*, 2004). As such, it is expected that triploids would retain a normal growth rate while the sexually mature diploids dedicate a significant energy portion for reproduction and thus the growth rate is compromised (Henken *et al.*, 1987; Mol *et al.*, 1994). The advantage of triploids over diploids is apparent for Salmonids of which sexual maturity is accompanied by poor meat quality, increase disease susceptibility and, in some species, changes of appearance (Mazeaud *et al.*, 1977). In the Pacific oyster (*Crassostrea gigas*), triploidy improves organoleptic quality and growth over the diploid (Nell, 2002). At present, triploid oysters are commercially produced (http://www.coastseafoods.com/triploid_oysters.html). In addition, sterility may be used as a tool for biological containment to avoid genetic contamination of the released or escaped fish as has been used for triploid rainbow trout (Sheehan *et al.*, 1999; Weber *et al.*, 2014) and grass carp in USA (Allen Jr. and Wattendorf, 1987; Chilton II and Muoneke, 1992). Some fish species which have a short reproductive cycle, for example, Nile tilapia (*Oreochromis niloticus*), reproduces in culture ponds and hence easily overpopulate the pond. Culture of triploid fish may solve the problem.

Potential applications of triploid for aquaculture in South East Asia

Aquaculture in South East Asia is important; some countries are world leading exporters of seafood from aquaculture, e.g. marine shrimp for Thailand, striped catfish for Vietnam (FAO, 2014). Triploidy is potentially useful in these species in various context, e.g. sterile Nile tilapia would efficiently solve the problem of overpopulated ponds while sterile genetically improved strain would avoid further unauthorized use of the strain. The following review collects the most updated information on triploid induction for the important aquaculture species in South East Asia countries.

Tilapia

South East Asian countries make a significant contribution to global Nile tilapia production with an annual production of approximately 1.3 million tonnes as of 2012 (FAO, 2014). Indonesia produces about 690,000 tonnes/year followed by the Philippines and Thailand (approximately 160,000 and 153,000 tonnes/year, respectively). Commercial culture of tilapia in South East Asian countries depends largely on Nile tilapia (*O. niloticus*), with a small portion of red tilapia which is a hybrid between *O. mossambicus* and *O. niloticus*. The production of Nile tilapia has been tremendously enhanced by culturing all-male fingerlings which are produced by hormonal sex reversal (Tayamen and Shelton, 1978). The culture of monosex fingerling avoids unwanted reproduction during the culture period. Furthermore, production is enhanced because males grow faster than females. However, due to food safety issues, although hormone residues were reported as below the safe standard in the product (Curtis *et al.*, 1991), alternative methods to avoid reproduction are required.

Triploidy has been induced in tilapia with the expectation that the sterile fingerlings will have improved growth rates over that of diploids. The triploid induction was done using various means, e.g. cold shock (Hussain *et al.*, 1991), heat shock (Pandian and Varadaraj, 1988; Hussain *et al.*, 1991; Puckhaber and Hörstgen-Schwark, 1996), and hydrostatic pressure shock (Hussain *et al.*, 1991) which gave up to 100% triploid. Recently, Pradeep *et al.* (2012a) induced triploidy in the red tilapia (*O. mossambicus* × *O. niloticus*) using heat shock (41°C, commenced 4 min after fertilization with shock duration of 3.5 min) which resulted in 89.7% triploids with a survival rate of 67%. The same group of researchers was able to increase the success rate of triploidy to 98.7% with 75.8% survival to yolk-sac stage by applying cold shock (9°C started at 4 min after fertilization for a period of 30 min) (Pradeep *et al.*, 2014). The authors suggested that the shock protocol should be optimized when different parental strains are used.

Triploid Nile tilapia outperformed their diploid counterparts (Brämick *et al.*, 1995). It is inconclusive whether triploid red tilapia grew better than their diploid counterpart, although Pradeep *et al.* (2012b) reported better growth performance of triploids over diploids. The result could have been biased by lower survival rate of triploids as compared to diploids. Interestingly, sex of the heat shocked triploid skewed towards maleness. There is a concern that application of this technique in commercial scale may be limited because tilapia has relatively low fecundity. Triploid induction in tilapia may be infeasible in terms of time and man power consumed as compared to the sex-reversal to maleness by hormone administration which is currently practiced (Pradeep *et al.*, 2012a, 2014).

Walking catfish

Walking catfish is very important to local

market in Thailand with annual production of 110,000 tonnes in 2012 of which a small portion was exported (FAO, 2014). Since the early 90s, the culture of Thai walking catfish (*Clarias macrocephalus*) gradually declined and eventually was completely replaced by the hybrid between female *C. macrocephalus* and male exotic *C. gariepinus* introduced from Africa. In an effort to improve growth of Thai walking catfish, triploidy was induced using cold shock (7°C, commenced at 0 min after fertilization and 25 min shock duration, 80% triploid production, Na-Nakorn and Lakhaantakun, 1993; 4°C applied at 2 min after fertilization for 15 min, 96% triploid production, Fast *et al.*, 1995). Growth performance obtained from these two studies was different. The triploid fish performed worse than the diploids in the first study while Fast *et al.* (1995) reported improved growth performance of triploids over diploids. The triploid induction performed on the congeneric species also gave varying results, namely, triploid *C. fuscus* grew better than diploid (Qin *et al.*, 1998) while similar growth performance was observed in diploid and triploid *C. gariepinus* (Henken *et al.*, 1987). It is possible that the difference of parental strains used in each study accounted for the varying results as suggested by Taniguchi *et al.* (1986) in common carp and in Atlantic salmon (Sacobie *et al.*, 2012). Recently our group found that strain, especially male parent, has significant interaction with ploidy on growth performance of triploid versus triploid Thai walking catfish (Chatchaipun *et al.*, personal communication).

At present, walking catfish production in Thailand is entirely of the Sharp-tooth catfish (*Clarias gariepinus*) introduced from Africa because of its relative high growth rate and hardiness. Although it is probably too late, concerns about impacts of this exotic fish on the environment should be taken into account. Na-Nakorn *et al.* (2004) and Senanan *et al.* (2004) reported genetic contamination

in the native *C. macrocephalus* by alleles from *C. gariepinus*, probably through back-crossing with the hybrid between these species that escaped from the culture ponds. Therefore, sterilization of *C. gariepinus* which, at present comprises almost 100% of the annual production of walking catfish in Thailand, by induction of triploidy may avoid further genetic contamination.

Triploidy was induced in *C. gariepinus* using cold shock at 5°C for 40 min started at 3 min after fertilization and yielded 95% triploid fish (Henken *et al.*, 1987). The growth comparison revealed no significant differences between diploids and triploids. However, after gutting the yield of triploid catfish was greater than that of diploids.

Overall, triploid walking catfish may not be suitable for aquaculture owing to low triploid yield while growth performance of triploids is not better than diploids.

Thai silver barb

Thai silver barb or Java barb (*Barbodes gonionotus*) is popular among consumers in Thailand and Malaysia. The overall production in South East Asia is 110,000 tonnes/year as of 2012 (FAO, 2014). Induction of triploidy of this species is expected to promote growth after sexual maturation which commences at about 8 months old while the culture period is about 12 months. Triploidy was induced in Thai silver barb by applying cold shock at 2°C started 0.5 min after fertilization for a duration of 10 min. Triploid yield was 72.5% (Koedprang and Na-Nakorn, 2000). However, the field trial revealed similar growth rates of diploid and triploid fish. Female triploids were completely sterile while a few spermatozoa were observed in the triploid testes. This study clearly showed that triploidy does not benefit the culture of Thai silver barb. Although growth of triploid may be improved by using different

parental strains (Taniguchi *et al.*, 1986; Sacobie *et al.*, 2012), the sterility of females may reduce market value because females with eggs are preferred.

Black tiger shrimp (*Penaeus monodon*)

Black tiger shrimp is one of the marine shrimp widely cultured in South East Asia with annual production exceeding 550,000 tonnes as of 2012 (FAO, 2014). Although it is a native species of this region, it has been largely replaced in aquaculture by the introduced Pacific white shrimp (*Litopenaeus vannamei*). The disadvantages of the tiger shrimp are slow growth rate and low disease resistance as compared to the genetically improved strains of Pacific white shrimp. Recently, genetic improvement programs have been initiated for *P. monodon* by private and governmental institutions. As such, there is a need to develop sterilization methods for the genetically improved shrimp to avoid unauthorized seed propagation. Triploidy has been induced in the tiger shrimp using cold shock (6.5-13.8°C; 7 min 30 sec post spawning; shock duration of 2, 4, 6 min after spawning) (Wood *et al.*, 2011) with varying results among spawns (success rates between 0-76.7%). The success rate was improved from 60.47% to 72.38% (up to post-larva stage) by using chemical shock (200 µM of 6-dimethylaminopurine, at 6 min 40 sec after spawning, a shock duration of 10 min) (Sellars *et al.*, 2012a). Sellars *et al.* (2012a) also performed growth trials and reported that triploidy reduced gonad development, sex ratio was 1 female: 1.625 male, growth of triploid and diploid was not different. Further study by Sellars *et al.* (2013) confirmed that male and female triploids were not able to reproduce. Using a Thai strain, Pongtippatee *et al.* (2012) induced triploidy in *P. monodon* by cold shock (8°C, 8 min post spawning, shock duration of 10 min). They achieved a triploid induction rate of 38%. The growth trial conducted in

a communal rearing tank showed that triploids grew faster than diploids. Sex ratio of triploid skewed towards femaleness (2 female: 1 male comparing to 2 female: 3 male of the diploid group).

The main obstacles prevent high success rates and hence the application of this technology on a commercial scale is asynchronous spawning. The solution would probably be using of an automated device that enable real-time detection of spawning and subsequent application of the shock protocol at precise timing (<http://www.sbir.gov/sbirsearch/detail/181343>; Mueandee *et al.*, 2013). Furthermore, an alternative approach could be production of tetraploid *P. monodon* to be used as a broodstock for mass triploid production. However, until now, attempt to induce tetraploids by applying cold shock were not successful (Foote *et al.*, 2012).

Future of triploid aquatic species

Research on induction of triploidy have been conducted for more than 40 years with limited success in terms of application to aquaculture with some exception, e.g. triploid rainbow trout (Weber *et al.*, 2014), triploid grass carp (Allen Jr. and Wattendorf, 1987; Chilton II and Muoneke, 1992) and triploid Pacific oyster (Nell, 2002). The main obstacles are egg mortality due to shocks and variation of growth performance even within species. Further studies are required aiming at optimizing shock protocols to get acceptable yields of triploids. In addition the rearing environment for triploids should also be optimized. In addition to aquaculture, studies on triploidy may enlighten knowledge on gene behavior.

In the context of aquaculture species of South East Asia, studies on triploidy are still limited. Owing to the large diversity of cultured species, the potential of triploid induction should be explored with specific objectives, for examples, to avoid aggressive

behavior of the giant river prawn (*Macrobrachium rosenbergii*), to establish biological containment of interspecific hybrids which are quite popular among farmers in the region, or to avoid unauthorized propagation of genetically improved strains. Triploid native oyster could also be also interesting, providing that the culture system of oyster is changed from using natural seeds to hatchery produced seed.

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