Polyploid fish - Principles, Importance and Applications in main marine aquaculture species

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Abstract :

In the important aquaculture farmed species (fishes, molluscs, crustaceans), controlling reproduction is important because the sexual maturation of the animals often has a major effect on the growth performance, survival, and organoleptic qualities of the final product. To overcome the downsides of sexual maturation, sterility can be induced via polyploiditism such as auto-triploidisation and allo-triploidization from tetraploids. Moreover, a polyploid state brings beneficial advantages for aquaculture. This paper is a review of the basic principles in ploidy manipulation such as for inducing triploidy in marine fishes along with some emphasis on salmonids, sturgeons and groupers. Both natural and artificial inductions are discussed with elaboration on potential advantages and disadvantages for each method. Finally, the triploid grouper induced from Epinephelus coioides (F) X Epinephelus lanceolatus (M) through hybridization is investigated.

Keywords: *Aquaculture, Grouper, Hybridization, Heterosis, Triploidy*

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Introduction

In the 1980s, aquaculture-oriented studies began to improve performances of farmed strains by chromosome manipulation techniques in various species of finfish and aquatic invertebrates, and became widely prevalent (Pandian and Koteeswaran, 1998a) (Purdom, 1983)[@yamazaki1983]. Chromosome manipulation is defined as a system of techniques to alter the number and combination of homo- and hetero-specific genome(s) orchromosome set(s). Several pioneer studies in fish species wereconducted from 1950s to 1970s to investigate the effect of ploidy elevation, as well as uniparental development on animals by scientists in the former Soviet Union (Neyfakh, 1956; Romashov, et. al., 1960;Romashov and Belyaeva, 1964), (Cherfas, 1975) , the United Kingdom (Purdom,1969; Purdom, 1972; Purdom, 1976), the United States (Stanley, 1976ab),Norway (Refstie, et. al., 1977), Hungary (Nagy et al., 2006)(Nagy et al., 1979) Japan (Ojima and Makino, 1978), and othercountries.

Triploidy is the condition in which somatic cells contain three sets of chromosomes; it arises from environmental changes or hybrid stabilization(Leggatt and Iwama, 2003a). In fishes, autotriploidy (with-inspecies) and allotriploidy (between species) are both caused by disturbances in ripening mechanisms of the eggs with a subsequent blocking of the extrusion of the second polar body (Flajšhans et al., 1993). Because of the economical and ecological benefits of generalizing such a practice to fish rearing, triploidy induction has become far more frequent and is now extended to many species of fresh- and seawater fishes from Cypriniformes to Pleuronectiformes. Indeed, fish farmers being, nowadays, more aware of the need to have efficient, environment-safe and sound rearing practices are more and more willing to use of reproductively sterile fishes. Although this practice sounds unable to guarantee 100% functional sterility of a treated population (Linhart et al. 2006)(Devlin and Nagahama, 2002) (Pandian and Koteeswaran, 1998b) (Linhart et al., 2006). This is a direct consequence of having an odd number of chromosome sets. The unpaired chromosome set leads to a failure of the normal pairing and crossing-over of homologous chromosomes that occurs during meiosis I (Benfey, 2011)

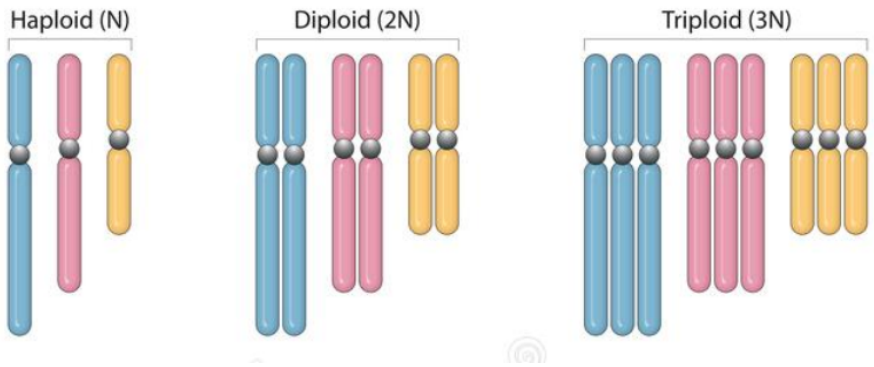

Figure 1: Different levels of ploidy

The first report of triploidization through hybridization in grouper was done by Huang et. al., (2014). Groupers (Serranidae, Epinephelinae) are distributed across all regions of the oceans, are globally one of the most commercially important groups of tropical marine fish and command high prices at markets (Beets and Hixon, 1994)(Shakeel and Ahmed, 1997)(Sluka and Reichenbach, 2000).

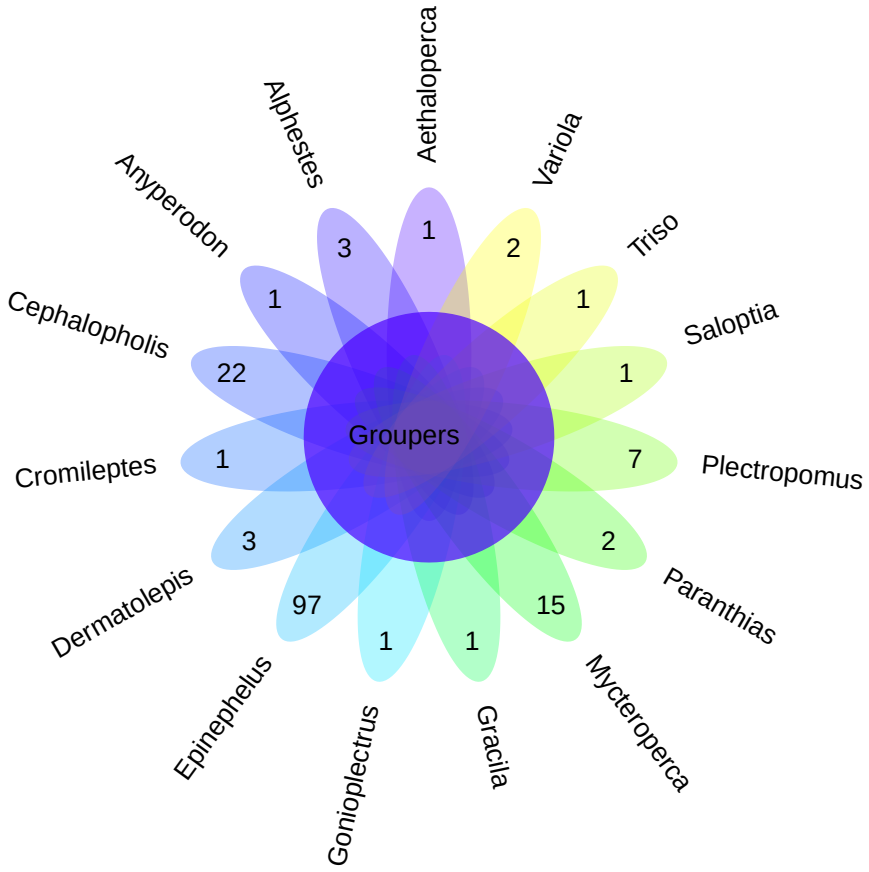

Figure 2: Distribution of the number of species by genus for the tribe Epinephilini (grouper)

Groupers comprise 15 genera and 159 species. (flower plot above)(R Core Team, 2016) The diversity of grouper species with different biological traits is an important resource for hybridization (Pierre, Gaillard,Prevot-D'Alvise, Aubert, Rostaing-Capaillon, Leung-Tack & Grillasca2008). Hybridizations between different groupers can generate new fish with hybrid vigor, which are of great value to the grouper-breeding industry. The Orange-spotted Grouper (Epinephelus coioides) and Giant Grouper (Epinephelus lanceolatus) are two of the most important species in the grouper industry. They form the majority of the groupers raised via aquaculture in Southeast Asia, especially in China. The Orange-spotted Grouper is more fecund and has a high survival rate but a slower growth rate. The Giant Grouper grows more rapidly but exhibits lower fertility and a higher rate of loss in artificial feeding stages before 2 years of age. Confirmation has been obtained through flow-cytometry, karyotyping and erythrocyte nuclei measurement.

The chromosome numbers of Epinephelus coioides, Epinephelus lanceolatus, diploid hybrid grouper are 48 and triploid hybrid grouper are 72 (Huanget. al., 2014).

Likewise, the first reported production of triploid Atlantic salmon (Salmo salar) was published over 60 years ago (Svärdson, 1945). It was another 30 years before further attempts were made to produce triploid Atlantic salmon, using methods that ultimately proved unsuccessful (Lincoln et al., 1974; Allen and Stanley 1979). Success followed 10 years later (Benfey and Sutterlin, 1984a; Bolla and Refstie, 1985; Johnstone, 1985; Johnstone et al., 1989; Quillet and Gaignon, 1990), leading to the pilot-scale evaluation of triploid Atlantic salmon for aquaculture in Scotland (Johnstone et al., 1991; Johnstone, 1993, 1996; McCarthy et al., 1996), Atlantic Canada (Friars and Benfey, 1991; Sutterlin and Collier, 1991; McGeachy et al., 1995, 1996; Pepper et al., 1996; O'Flynn et al., 1997; Benfey, 2001; Friars et al., 2001), Tasmania (Jungalwalla, 1991), the USA (Galbreath et al., 1994; Galbreath and Thorgaard, 1995), Ireland (Cotter et al.,

2000; Wilkins et al., 2001; Cotter et al., 2002) and Norway (Oppedal et al., 2003).

Figure 3: A salmon jumping

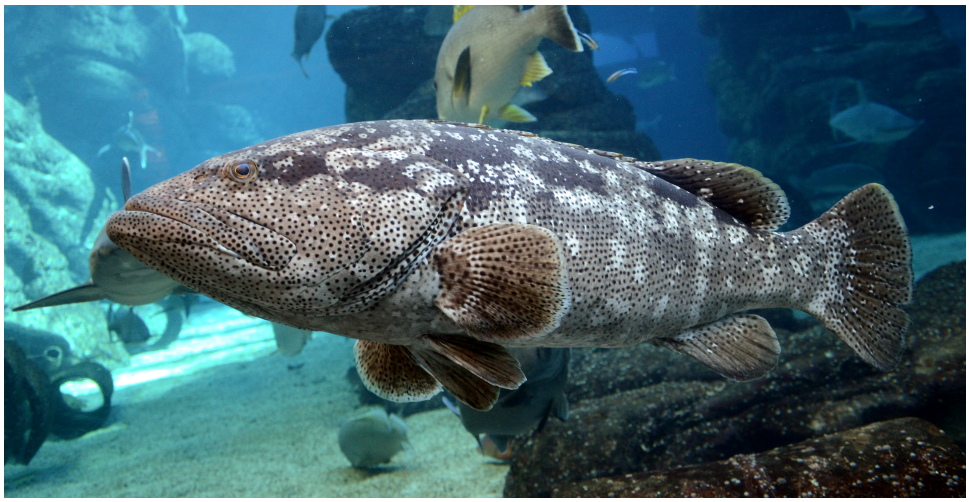

Figure 4: The malabar grouper (Epinephelus malabaricus)

This paper presents the general mechanism of polyploidy and the difference between the mechanisms of auto-triploidy and allo-triploidy with emphasis on the salmon and grouper taxon. Then, advantages and disadvantages of the various methods for the production of triploids are also discussed to provide further information on the triploidization through hybridization in grouper. Finally, the last section summarizes the current knowledge in triploid salmon and some perspectives for the triploid groupers in aquaculture industry.

Status and economic importance of salmon in aquaculture

Pacific salmon have been an important food source and trade good for native peoples of the North Pacific rim since prehistoric times (Newell 1994). Two species, Chinook and coho, are increasingly used in marine aquaculture with net pen production of these species being almost entirely confined to Chile (primarily coho) and New Zealand (Chinook) (Criddle and Shimizu, 2014). The global production of salmon for the main species was made from the data accessible by the FAO. (FAO, 2018)

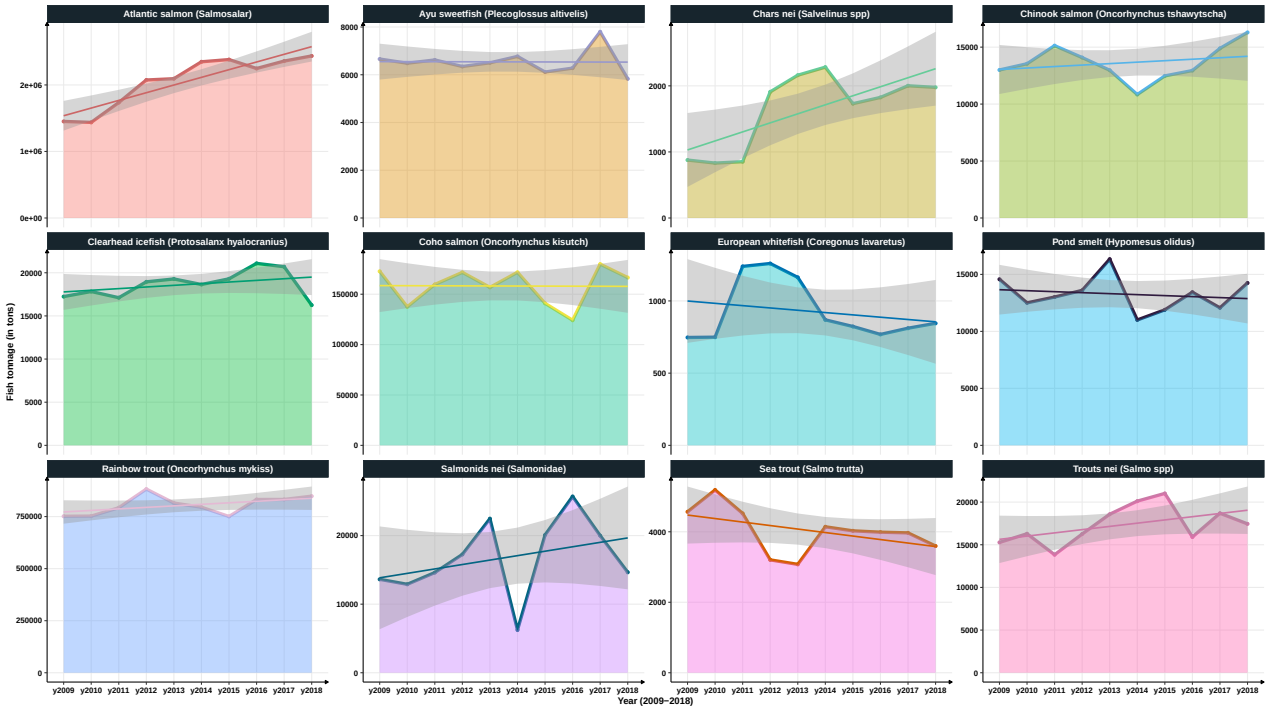

Figure 5: Global production of salmons and trouts

In the fisheries, the most desired traits to improve in salmons are the quantitative traits such as the mass of the fillet but also qualitative traits such as the fillet color. The main traits are cited below: (data from (Tsai et al., 2015))

Table 1: Important breeding traits in the Atlantic salmon

Traits	Values		
Trait	Sample.Size	Mean.SD	Heritability.SE
Harvest Weight	1524	2.57 (0.63)	0.52 (0.05)
Gutted Weight	1616	2.35 (0.58)	0.53 (0.05)
Gutted Yield	1447	0.92 (0.02)	0.04 (0.01)
Deheaded Weight	1604	2.06 (0.52)	0.52 (0.05)
Fillet Weight	1516	1.70 (0.42)	0.53 (0.05)
Fillet Yield	1363	0.66 (0.04)	0.05 (0.02)
Fat Percentage	1679	12.2 (5.58)	0.18 (0.03)
Fillet Colour	1322	29.0 (0.73)	0.14 (0.03)
Head Weight	1475	0.32 (0.08)	0.21 (0.03)a
Gut Weight	1447	0.42 (0.08)	0.30 (0.04)a
Body Waste Weight	1426	0.33 (0.12)	0.15 (0.02)a
Total Waste Weight	1422	0.65 (0.17)	0.32 (0.04)a

Table 2: Nomenclature and distribution area of the three genera of salmonids

Taxonomy		Location
Genus	Scientific.and.vernicular.names	Native.distribution
Oncorhynchus		
	Oncorhynchus nerka (sockeye salmon)	North Pacific Rim: Japan: Columbia River
	O. gorbuscha (pink salmon)	North Pacific Rim: Korea to California
	O. keta (chum salmon)	North Pacific Rim: Korea to California
	O. tshawytscha (chinook salmon)	North Pacific Rim: Northern Japan to California
	O. rhodurus (amago salmon)	Southern Japan
	O. masou (masu salmon)	Japan, parts of Russia
	O. kisutch (coho Salmon)	North Pacific Rim: Northern Japan to California
	O. mykiss (steelhead and rainbow trout)	North Pacific Rim: Mexico to Russia and Interior of N. America mainly east of Rocky Mountains
	O. clarki (cutthroat trout)	West Coast of N. America, Alaska to California. Some interior western strains.
Salvelinus		
	Salvelinus alpinus (Arctic charr)	Circumpolar, and southward as relict populations
	S. malma (Dolly Varden charr)	Western North America and Eastern Asia
	S. confluentus (bull trout)	North Western North America
	S. fontinalis (Eastern brook charr)	Northeastern North America
	S. namaycush (lake charr or lake trout)	Northern Canada and southward relict populations
Salmo		
	Salmo salar (Atlantic salmon)	North Atlantic Ocean and surrounding land masses and water bodies
	Salmo trutta (brown trout)	European continent, U.K, Ireland

Key societal drivers that change how humans value salmon include for instance urbanisation, which affects cultures and practices in how humans relate to nature, increasing interests and social norms towards non-consumptive use, and demographic changes such as an aging population in many countries (Arlinghaus et al. 2002). Another driver for changes in the value of wild Atlantic salmon, especially its value as food, is the rapid expansion of the salmon farming industry since the 1980s. Figure 1 shows the annual production of over one and half million tonnes of farmed salmon in the North Atlantic area (ICES, 2018). It has provided an increasingly cheap substitute for wild salmon, though there is still a market for wild salmon.

In Japan, important marketing is done toward a natural salmon. The salmon hatcheries allow the salmons to spend most of their lifecycle at-large in the natural environment, free of pharmaceuticals and artificial hormones, and the high production make it reliably available. Moreover, salmon processed in Japan meet the highest quality standards. To maximize the economic value of Japanese salmon fisheries, it is important to develop local strategies for hatchery enhancement that take into account global market trends related to eco-labeling and product forms (Engle and Quagrainie 2006). It is anticipated that additional expansion of domestic salmon in the Japanese market will continue to depend, in part, on export growth.

Status and economic importance of grouper in aquaculture

Groupers are economically valuable, making up an important part of the catch of sport and artisanal fishers throughout their distribution (Seng, 1998). About 40 species of groupers occur in the Philippines, where they are caught by small-scale fishermen with hook and line, bamboo traps, or dip net from estuaries and coral reefs (Kohno et al., 1988). Global grouper production increased dramatically in recent years, with 60,774, 99,378, 163,093, and 198,690 mt in 1990, 2000, 2005, and 2007, respectively (FAO, 2005a,b, 2009).

Historically the red grouper, E. morio constituted the most important finfish fishery in the Mexican territorial waters within the Gulf of Mexico (Albanez- Lucero and Arreguín-Sánchez, 2009). Growth of the fishery has been observed from 1947 to 1972, with the highest yield of about 21,000 mt in the year 2000s (Burgos-Rosas and Pérez-Pérez, 2006). Since then, the wild stock appears to have been depleted; by 2004, the yields were less than 6000 mt. Present stocks are about a third of those estimated in the early 70 s (Doi et al., 1981). In 1995, feral production of groupers reached 27,359 mt from the Philippines, Malaysia, Taiwan, and Thailand; while the total grouper production from the entire South China Sea area yielded 1348 mt from brackish water aquaculture and 771 mt from mariculture (SEAFDEC, 1997). However there is an insatiable demand for groupers as luxury protein. For instance in Spain, dusky grouper is highly appreciated by consumers owing to the excellent properties of the meat. This species is frequently sold around 30–40 €/kg in the marketplace (Asensio et al., 2009).

Aquaculture of groupers is carried out in tropical and subtropical areas throughout the world, but most production is from Asia, with three countries responsible for an estimated 92% of global production: China (65% of total production), Taiwan Province of China (17%) and Indonesia (11%) (Rimmer and Glamuzina, 2017). Based on national production data, almost 155 000 tonnes of grouper were produced in 2015 with a total value of USD 630 million (Fig. 1) as reported by FAO (2017).

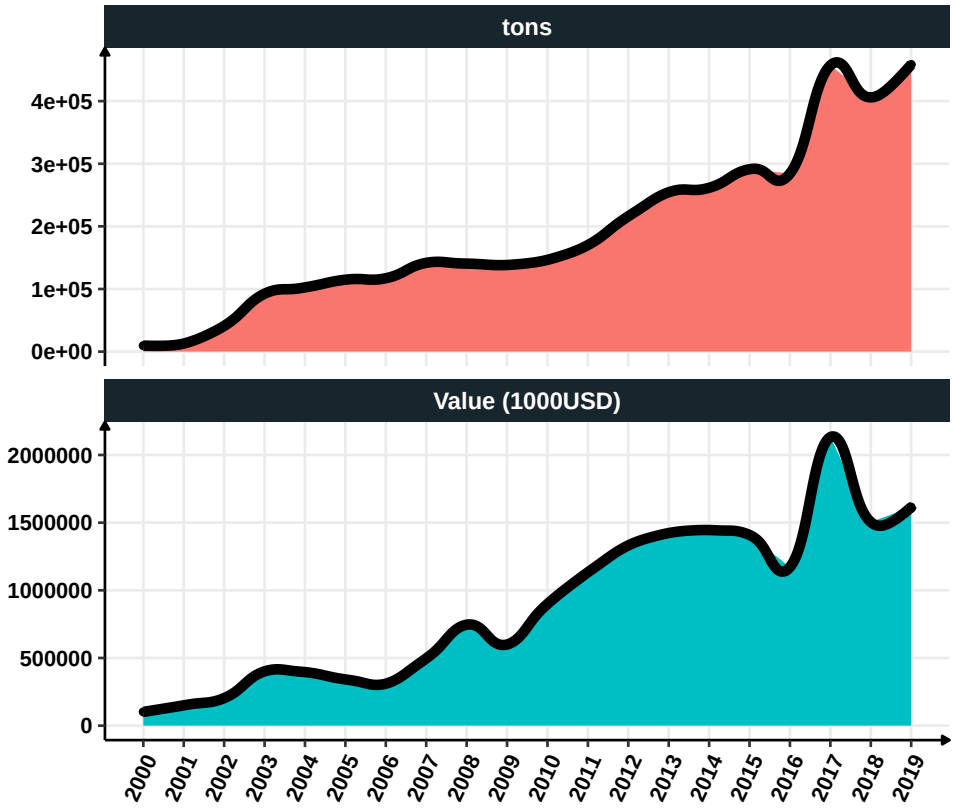

Figure 6: Global quantity and value for the grouper aquaculture industry - data from FishSTAT

China produces a variety of grouper species. Liu and Sadovy de Mitcheson (2008) listed 11 species that are routinely farmed and this list was compiled before the recent surge in production of hybrid groupers. Hong and Zhang (2003) listed six grouper species that are routinely produced in hatcheries, but note that annual production of five species is <100 000 fingerlings per annum. Because of constraints to expansion of aquaculture in inshore areas, China has begun to develop offshore farming systems using larger circular cages constructed from HDPE (Chen et al. 2007; Kongkeo et al. 2010). These larger cages are used to culture fast-growing species like giant grouper E. lanceolatus (Kongkeo et al. 2010). The development of recirculating production systems for grow-out of groupers in Hong Kong is still limited. Liu and Sadovy de Mitcheson (2008) reported that these systems have been used to culture E. lanceolatus and Cromileptes altivelis.

Another major producer of both fingerlings and market-sized grouper is Taiwan PoC, which supplies to countries throughout Asia. The technology for production of marine finfish, including groupers, has been reviewed by Liao et al. (2001) and Su et al. (2008). Grouper farming has been expanding in Taiwan PoC, with land area used for grouper farming increasing from 1235 ha in 2003 to 2336 ha in 2011 (Ahn et al. 2014). The development of the 2010 Economic Cooperation Framework Agreement (EFCA) between Taiwan PoC and China resulted in an easing of trade restriction on grouper imports to China because of inclusion of grouper on the 'Early Harvest List' of the EFCA (Ahn et al. 2014). The impact of this was an increase in grouper exports to China, reaching 7877 tonnes valued at around USD 102 million in 2011, and an increase in price from USD 10.11 in 2010 to USD 12.96 in 2011 (Ahn et al. 2014).

The development of grouper aquaculture in Indonesia has been strongly supported by the Indonesian government, through the Ministry of Marine Affairs and Fisheries (MMAF). The development of hatchery technology for a range of grouper species was undertaken by the Institute for Mariculture Research and Development Gondol, and much of the research and development effort was conducted in collaboration with Japanese and Australian research agencies (Sugama et al. 2008). Grouper hatcheries have also proliferated in East Java (around Situbondo) and in southern Sumatra (Lampung; Sugama et al. 2008). Expansion of grouper hatchery production beyond northern Bali has been effected through a national network of technical implementation units (TIUs) established by MMAF. The TIUs provide local support for aquaculture technologies, including extension and technical support services, and importantly, provide a source of eggs for hatcheries that do not have access to broodstock (Hishamunda et al. 2009; Rimmer et al. 2013a). Development of grouper hatcheries in East Java and in southern Sumatra has been supported by TIUs at Situbondo and Lampung, respectively. An economic evaluation of grouper aquaculture in Indonesia (Riau Islands, Lampung, East Java and Bali) showed that, for tiger grouper (Epinephelus fuscoguttatus), small farms (7–15 cages) provided negative economic indicators, while medium farms (20–28 cages) provided only marginal positive indicators, and only large (48+ cages) farms culturing tiger grouper provided strongly positive economic indicators (Afero et al. 2010). All farm sizes culturing C. altivelis provided positive economic indicators, but these improved as farm size increased (Afero et al. 2010).

The tiger grouper has for some years been the most popular species for grow-out culture in Indonesia. More recently, two hybrid groupers have proven to be popular: E. fuscoguttatus x E. polyphekadion (known in Indonesia as 'kerapu cantik') and E. fuscoguttatus x E. lanceolatus ('kerapu cantang'). Indonesian grouper farmers report that both hybrids are more robust and less prone to disease than tiger grouper. Fish are generally stocked to reach a final density of 15–20 kg/m3 (Kongkeo et al. 2010), although this is often much lower during the early stages of grow-out. The duration of culture is about 9–12 months for Epinephelus spp., 12–14 months for Plectropomus leopardus and 18–24 months for C. altivelis (Kongkeo et al. 2010).

General principles and methods for induction of polyploidy including triploidy

Polyploidization

Polyploid fish are created by the process of polyploidization. The Polyploidization is a whole genome duplication. This mechanism is able to "instantly" give birth to new species. (Vallejo-Marín et al., 2015) While polyploidy is rarer in animals than in plants, it is still possible, infact, in animals the doubling of chromosome number during division can occur and polyploids individuals can be viable. The fish are not real hermaphrodite (simultaneous) and therefore cannot self-fertilize. After whole genome duplication (polyploidization) If the fish is triploid it will probably never give birth to a tetraploid because the individuals will have uneven number of sex-producing genes and become unfertile.(determination through absolute quantities of the sex chromosome) (Muller, 1925)

The duplication event is an addition to a set of chromosomes leading to a expansion of the chromosome sets for instance: 2n becomes becomes 4n.(pol, a) The polyploidy can be repeated along the fish taxon: Acipenseriformes, Cypriniformes, Characiformes, Gymnotiformes, Perciformes, Salmoniformes, and Siluriformes. (Mei and Gui, 2015; Taylor et al., 2003) This repetition is the result of whole genome duplication. (Glasauer and Neuhauss, 2014)

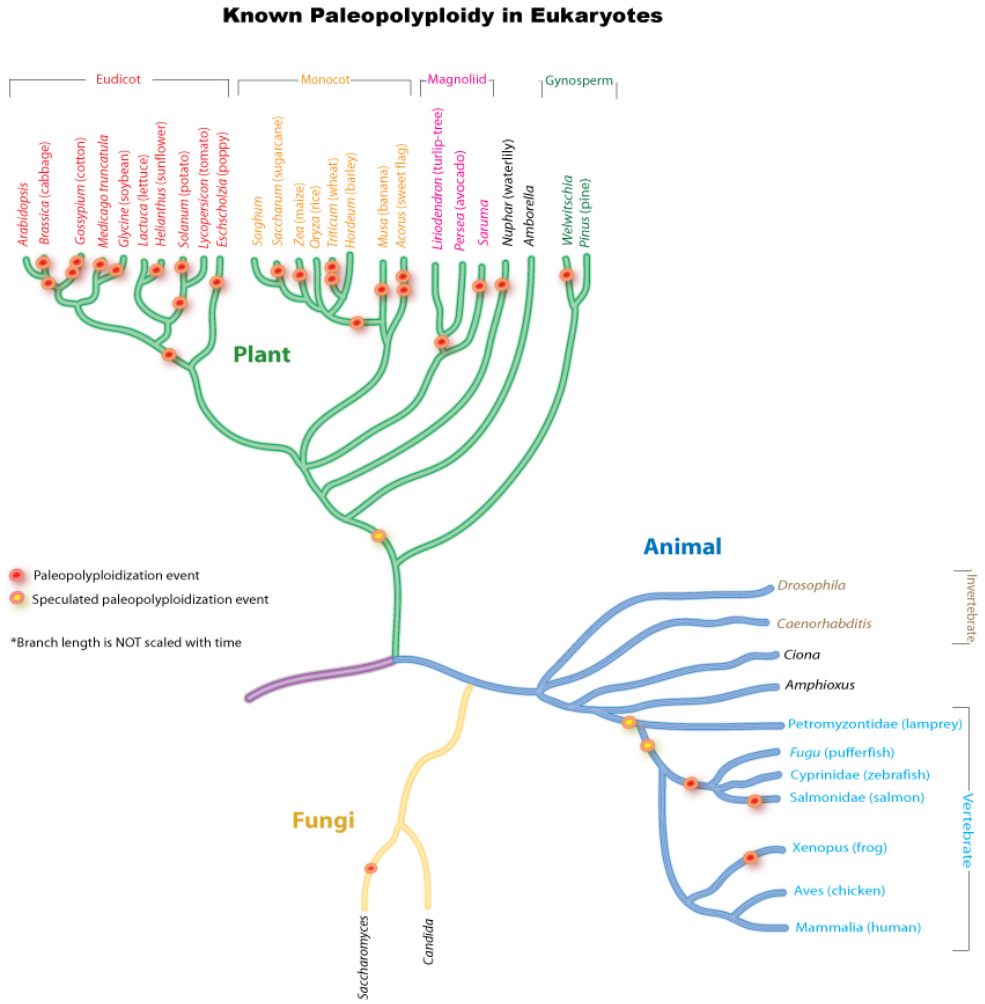

Figure 7: Different polyploidization events in eukaryotes: About 500 to 600 million years ago (Mya) the vertebrates common ancestor experienced two whole genome duplication (WGD) events. All teleosts may have experienced a thrid WGD event at approximately 320 to 350 Mya (hypothesis).

The 2R WGD events (Dehal and Boore, 2005) and the 3R WGD event (Vandepoele et al., 2004)(A. et al.,

2006) are polyploidization events.

Natural polyploids

Some examples of natural polyploid events in the marine and freshwater fishes:

The Carps such as *Schizothorax prenanti* can have as much as 148 chromosomes and a ploidy of 6N (hexaploidy) and are due to successive and late recombinaison events (Prussian carp) 18.49 Mya and 0.51 Mya. (Li et al., 2014a; Yu et al., 1987)

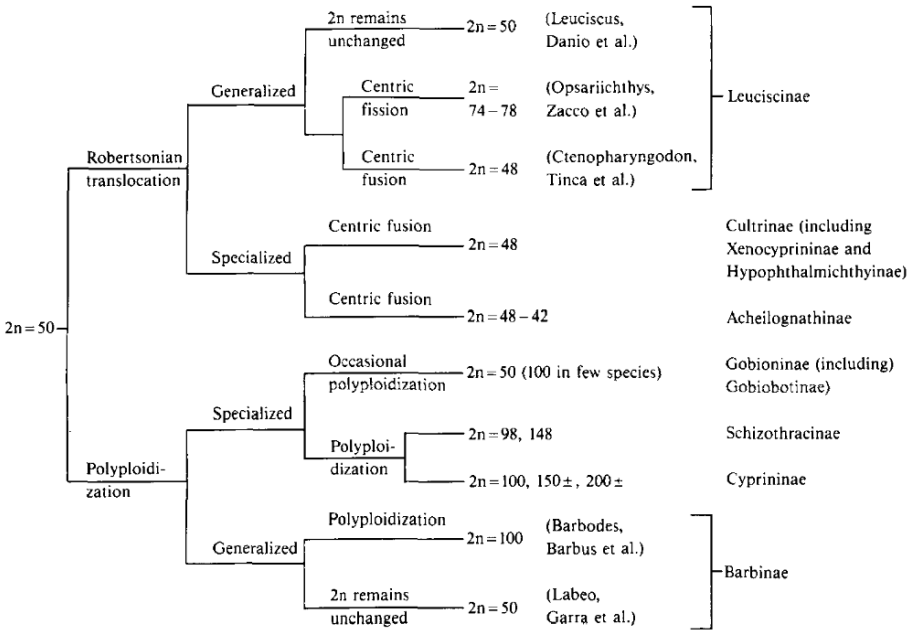

Figure 8: Scheme of the karyotypic evolution and phylogenetic relationship of cyprinid fishes

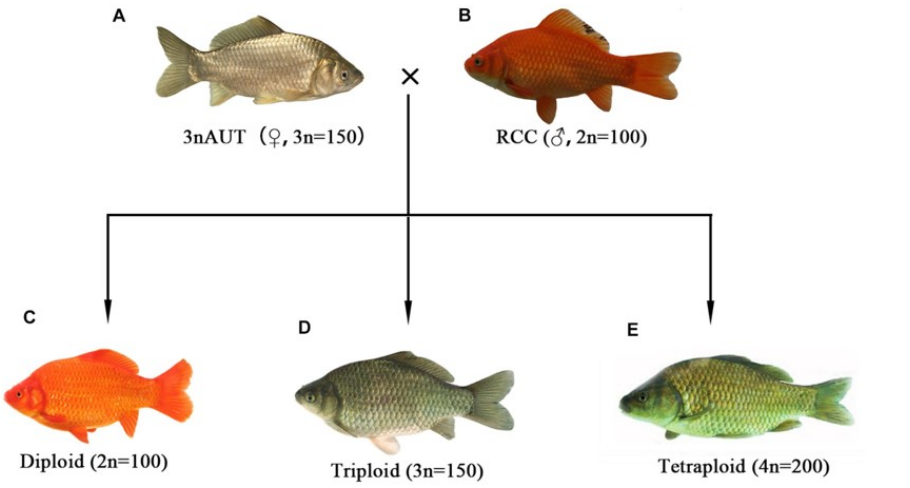

Figure 9: A cross between two species can produce a change in the ploidy levels (F1 hybrids)

Sturgeons (Acipenseridae)

The sturgeons of the family Acipenseridae experienced at least 3 polyploidization events,(Vasil'ev et al., 2010; Rajkov et al., 2014) leading to a huge amound of chromosomes found by karyotyping. For instance the Sakhalin sturgeon *Acipenser mikadoi* includes 262 ± 4 chromosomes. Concerning the polyploidization events, we believe that 2 polyploidization events occurred in the Atlantic species group: The first one in the common ancestor of a tetraploid 4N Atlantic species. The second one in the origin of hexaploid A.breviro-strum. However, the evolution of polyploidy in A.brevirostrumis probably more complex, as proposed by (Fontana et al., 2008) and also likely to involve allopolyploidy. In conclusion, at least 3 polyploidization events occurred in the evolution (Figure1), but additional hybridization events cannot be refuted. (Peng et al., 2007)

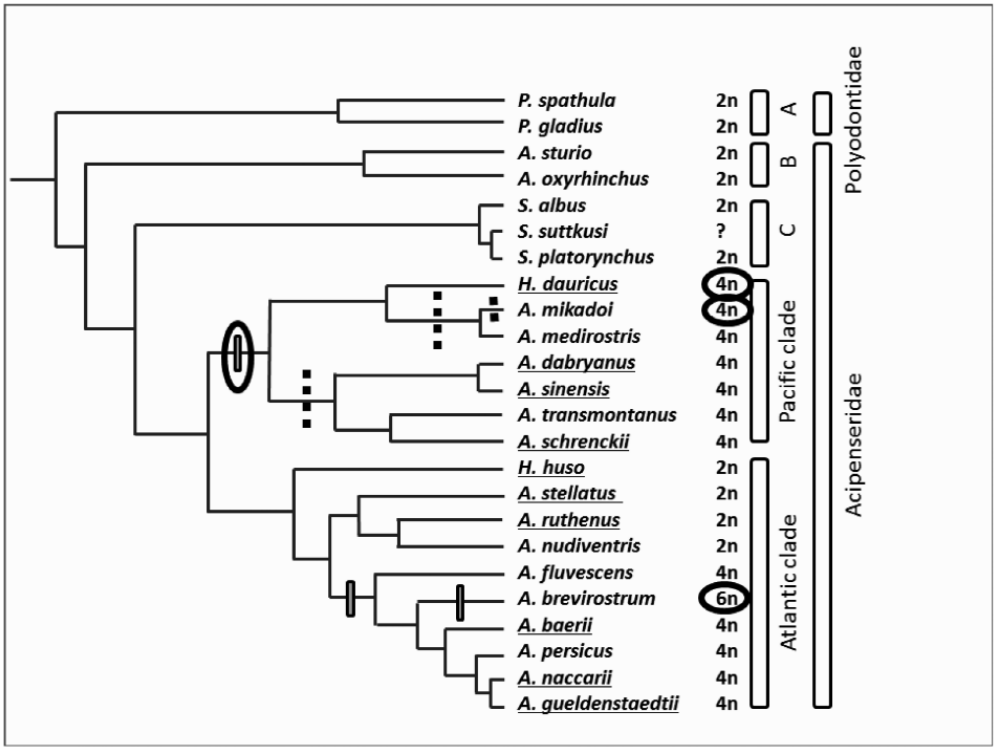

Figure 10: Phylogenetic tree modified from Peng etal. (2007): the underlined taxa have been investigated in this study, the ellipses indicate new information, the vertical bars are deduced duplication events, and the dotted vertical lines indicate duplication events that are no longer necessary to invoke. A=genus Pseudoscaphirynchus, B=sea sturgeons clade, and C=genus Scaphirynchus. Recent data are ploidy levels in Huso dauricus (4n), Acipenser mikadoi (4n) (Vasil'ev etal. 2009), and Acipenser brevirostrum (6n) (Fontana etal. 2008) with presumable duplication events, proposed by Vasil'ev etal. (2010) and supported by this study, mapped on the branches. However, evolution of polyploidy in A.brevirostrum is probably more complex, as proposed by Fontana etal. (2008) and likely also involves allopolyploidy.

The salmonids

The salmonids are mostly marine water fishes such as the atlantic salmon *Salmo salar*. In the cultured salmonid species, hybridization improves greatly the survival especially if triploidy is co-induced (Scheerer and Thorgaard, 2011a). Polyploidy is therefore beneficial to hybrids the salmonids are either diploids 2N or triploids 3N they can form spontaneously by WGD tetraploid 4N or hexaploid 6N individuals. (figure below)(Leggatt and Iwama, 2003b)(Allendorf and Thorgaard, 1984)

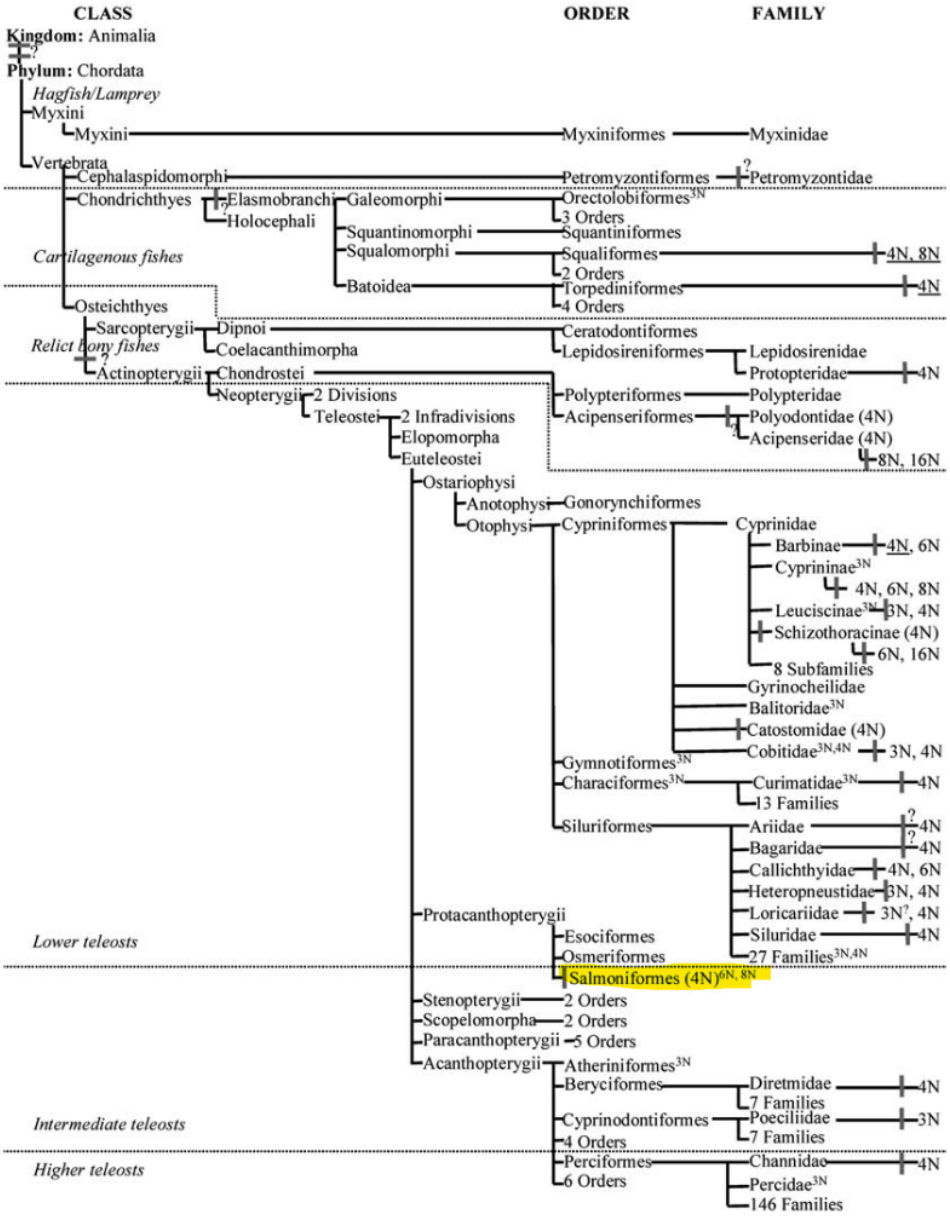

Figure 11: The occurrence of polyploidy in the fishes. Black bars denote polyploid event(s); placement before a group name indicates the entiregroup is polyploid and placement after a group indicates only some of the group is polyploid. Levels of polyploidy are indicated by N (i.e.,4N = tetraploid), spontaneous polyploid individuals are indicated by superscripts, and polyploid events that are in question are indicated by"?". The taxonomic classification of the fishes was taken from Moyle and Cech (1996). The further classification of the Family Cyprinidaewas taken from Ruiguang et al. (1986). The teleosts are divided into lower, intermediate, and higher groups according to the classification byGosline (1971).

Autopolyploids

The autopolyploid individual get a double chromosome set from a single ancestral species (duplicate), it is possible because of a genome by non-disjunction of the gametes (gametic non-reduction). (Madlung, 2013) In the case of the creation of a viable organism, we can observe advantages, the duplication leads to allele compensation: masking the deleterious recessive mutations. It insures against the loss off fitness. (Gu et al., 2003)

Allopolyploidy

The allopolyploid individual get an the extra chromosome set from another species and not from an ancestral species, the allopolyploids are very likely to become unfertile.

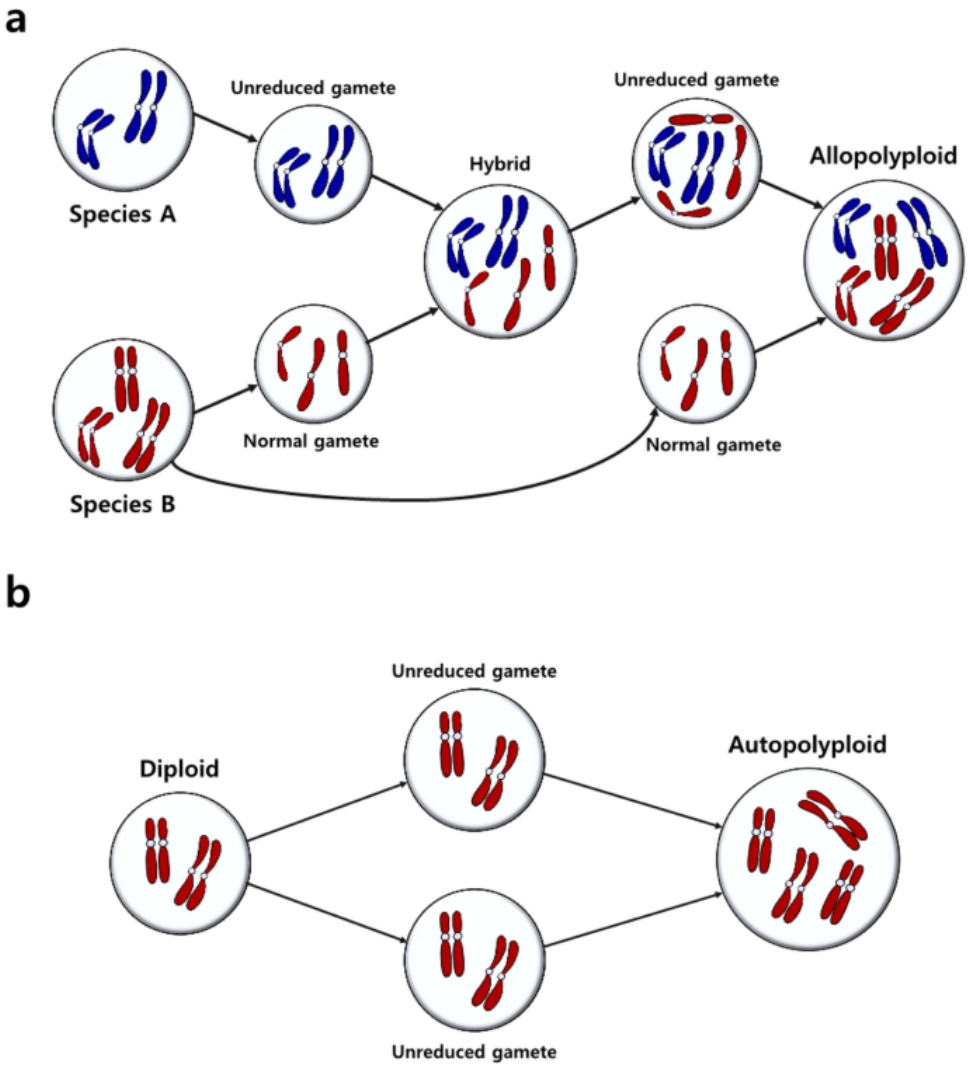

Figure 12: Whole genome duplication (WGD) of autopolyploids and of allopolyploids

Table 3: Natural polyploids of aquatic animals used in aquaculture

Taxonomy		Genetics	
Family	Species	Chromosome.number	Ploidy
1. Fish			
Polydontidae	Mississippi paddlefish (Polyodon spathula)	120	4N
Acipenseridae	Russian sturgeon (Acipenser gueldenstaedtii)	236 ± 2	8N
	Chinese sturgeon (A. Sinensis)	240	8N
	Starlet sturgeon (A. Ruthenus)	116 ± 4	4N
	Amur sturgeon (A. Schrenckii)	238 ± 8	8N
	Siberian sturgeon (A. Baerii)	245	8N
	Danube sturgeon (A. Gueldenstaedtii)	240-264	8N
	White sturgeon (A. Transmontanus)	240-265	8N
	Adriatic sturgeon (A. Naccarii)	240-266	8N
	Kaluga (Huso dauricus)	268 ± 4	8N
	Beluga (H. Huso)	120	4N
Cyprinidae	Common carp (Cyprinus carpio)	100	4N
	Crucian carp (Carassius auratus)	100	4N
	Goldfish (C. Auratus)	100	4N
	Gibel carp (C. Gibelio)	156 or 162	6N
	Barbel (Barbus barbus)	100	4N
	Algerian barb (B. Callensis)	100	4N
	Barbodes (Spinibarbus denticulatus)	100	4N
	Schizothorax prenanti	148	6N
	S. Davidi	98	4N
Cobitidae	Pond loach (Misgurnus anguillicaudatus)	75, 100, 150	3N, 4N, 6N
Catostomidae	Bigmouth buffalo (Ictiobus cyprinellus Valenciennes)	100	4N
Salmonidae	European whitefish (Coregonus lavaretus)	80 or 81	4N
	Peled (Coregonus peled)	74	4N
	Arctic char (Salvelinus alpinus)	78	4N
	Brook trout (S. Fontinalis)	84	4N
	Atlantic salmon (Salmo salar)	54-58	4N
	Brown trout (S. Trutta)	78-84	4N
	Sevan trout (S. Ischchan)	80-82	4N
	Chinook salmon (Oncorhynchus tshawytscha)	68	4N
	Chum salmon (O. Keta)	74	4N
	Coho salmon (O. Kisutch)	60	4N
	Rainbow trout (O. Mykiss)	58-64	4N
	Golden trout (O. Mykiss aguabonita)	58	4N
	Masu salmon (O. Masou)	66	4N
	Sockeye salmon (O. Nerka)	57 or 58	4N
	Danube salmon (Hucho hucho)	82	4N
	European grayling (Thymallus thymallus)	102	4N
Osmeridae	Pond smelt (Hypomesus olidus)	56	4N
Salangidae	Clearhead icefish (Protosalanx hyalocranius)	56	4N
Clariidae	Philippine catfish (Clarias batrachus)	100	4N
Heteropneustidae	Stinging catfish (Heteropneustes fossilis)	87, 116	3N, 4N
Loricariidae	Suckermouth catfish (Hypostomus plecostomus)	54	3N
Siluridae	Wallago catfish (Wallago attu)	86	4N
	Wels catfish (Silurus glanis)	60	3N
2. Crustaceans			
Cambandea	Red swamp crayfish (Procambarus clarkii)	188-192	4N
	Japanese crayfish (Cambarus japonicas)	196 or 200	4N
Nephropidae	Ridge-back lobster (Nephropsis carpenter)	152	4N
	Japanese lobster (N. Japonicus)	136 or 164	4N
3. Molluscs			
Buccinidae	Whelk (Neptunea cumingi)	60	4N

Artificial polyploids

Concerning the triploid state in fish, it can occur naturally but it could also be a result of manipulation (i.e. an artificial induction). In either case, triploids animals are formed by sexual reproduction and during meiosis either naturally or artificially.
Meiosis is the process by which a single cell divides twice (meiosis I and meiosis II) to produce 4 cells containing equal amounts of genetic information. (Rhoades, 1961)(pol, b)(May and Delany, 2015)

As a reminder, meiosis I consists of the duplication of the homologous chromosomes (leading to a ploidy of 2n) followed by the extrusion of one pair of chromosomes (leading to a ploidy of (n) in the form of a first polar body. In meiosis II the process repeats prior to the fertilisation and a second polar body should be extruded. Although, in the induction of triploidy the extrusion of the second polar body is blocked. Once the sperm cell and the fish egg are put in contact, the fertilization begins with the acrosomal reaction. The sperm genetic information enters the egg and the meiosis II can continue. If one decides to block the extrusion of the second polar body using hydrostatic pressure (increasing the pressure on the activated egg), or by cold or heat shock treatment then the zygote will keep it's triploid state (see the diagram below). (Zhou and Gui, 2017)

Direct induction methods

Cold shock induction

Using cold temperatures for meiosis II induction of nondisjunction. It is commonly used in freshwater shrimps such as *Macrobrachium rosenbergii*

Chemical induction

Induction of triploids in meiosis I and II with 6-dimethylaminopurine (6-DMAP), cytochalasin B, polyethylene glycol, 6-dimethylaminopurine (6-DMAP), caffeine, and colchicine, are alsoused to disrupt poly body extrusion, but they are generally oflimited use due to their chemical toxicity. (Piferrer et al., 2009a)

Heat shock induction

Example of the salmonids: All possible hybrid crosses between brook (Salvelinus fontinalis), brown (Salmo trutta), and rainbow (Salmo gairdneri) trout were made and a portion of the fertilized eggs from each mating were heat shocked to induce triploidy. Within a species, triploids generally showed poorer survival to the initiation of feeding than diploids. In most crosses, however, triploid hybrids showed much better survival than diploid hybrids. The triploid tiger trout (brown × brook) hybrid showed the most potential of the hybrids tested. Induced triploidy could be a useful general method for increasing survival in interspecific fish hybrids. (Scheerer and Thorgaard, 2011b)

Hydrostatic pressure shock

With the temperature shock, the hydrostatic pressure shock is the most common physical method for polyploidy induction. Triploidy or tetraploidy inducing techniques and will depend on the variables such as the timing, intensity and duration, to guide polyploidy induction infish and shellfish it is recommended to follow the mainstream procedures and protocols.

Table 4: Artificial or synthetic polyploids used in aquaculture

Taxonomy		Genetics	Manipulations	
Family	Species.or.variety	Ploidy	Inducing.or.synthetized.methods	References
1.Fish				
Cyprinidae	Grass carp (C. Idella)	3N	Hydrostatic pressure treatment	
	"xiangyun" crucian carp	6N	Interspecific hybridization and	(Chen et al., 2009)
	"Xiangyun No. 2" crucian carp	6N	Interploid crossing	
	"Changfeng" gibel carp (Synthetized allopolyploid)	8N	Incorporating an alien sperm genome	(Li et al., 2016)
Cobitidae	Cyprinid loach (M. Anguillicaudatus)	3N	Interploid crossing	(Hulata, 2001)(Piferrer et al., 2009b)
Salmonidae	Rainbow trout (O. Mykiss)	3Na	Thermal shock or Hydrostatic pressure treatment	(Solar et al., 1984) (Hulata, 2001)
			Interploid crossing	(Piferrer et al., 2009b)
	Brown trout (S. Trutta)	3Na	Hydrostatic pressure treatment	
	Brook trout (S. Fontinalis)	3Na	"	
	Atlantic salmon (S. Salar)	3Na	" also NO, Freon, Cytochalasin B	
	Chinook salmon (O. Tshawytscha)	3Na	"	
	Arctic char (S. Alpinus)	3Na	"	
	Amago salmon (O. Rhodurus)	3Na	"	(Arai, 2001)
	Masu salmon (O. Masou)	3Na	"	(Arai, 2001)
	Coho salmon (O. Masou)	3Na	"	
	Allotriploid rainbow trout amago .. (X) Salmon hybrids	3Na	Interspecific hybridization and Heat-shocked	
	Rainbow trout Japanese charr hybrids	3Na		(Arai, 2001)
Siluridae	Thai walking catfish African .. (X) Catfish hybrids	3N	Interspecific hybridization and Heat-shocked	(Na-Nakorn et al., 2004)
Plecoglossidae	Ayu (P. Altivelis)	3N	Thermal shock or hydrostatic	(Arai, 2001)(Hulata, 2001)(Piferrer et al., 2009b)
Paralichthyidae	Hirame (P. Olivaceus)	3N	Pressure treatment	(X et al., 1996)
2. Shellfish				
Ostreidae	Pacific oyster (C. Gigas)	3N	Thermal shock, hydrostatic pressure Treatment or interploid crossing	
Pectinidae	Catarina scallop (Argopecten ventricosus)	3N	Cytochalasin B	

[1] *a* Triploids (3N) are defined as the natural tetraploid species are regarded as diploids

[2] *(X)* Animal crossing

Triploid and tetraploid manipulation in fish

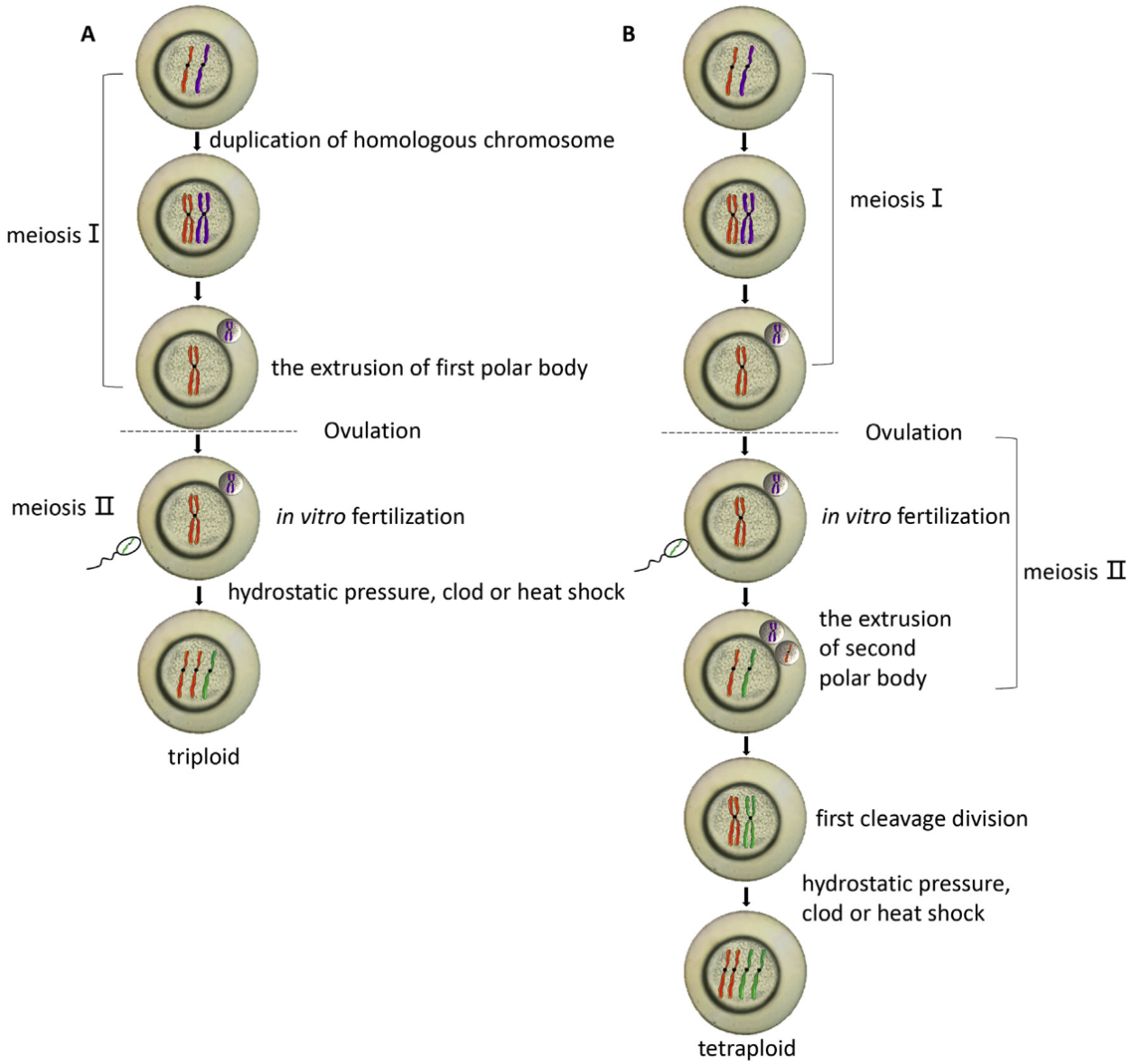

Figure 13: A schematic diagram of triploid or tetraploid manipulation infish. (A) Induction of artificial triploid by blocking the extrusion of the second polar body through hydrostaticpressure, cold or head shock. (B) Induction of artificial tetraploid by suppression of thefirst cleavage division through hydrostatic pressure, cold or head shock. The ovulated matureeggs are arrested at the metaphase stage of meiosis II.

Extract from (Hershberger and Hostuttler, 2007) *"Since the 1990s, triploid Pacific oysters, with increased growth rate and reduced gonadal development which enables them to be marketed all year with a high quality product, have become an important aquaculture industry. Currently, nearly 50 percent of cultured Pacific oysters in USA are triploids (Table 2). Theoretically, the utility of fertile tetraploids to produce infertile triploids is not only more efficient and more predictable than the direct induction of triploidy through thermal or hydrostatic pressure shock, but also provides the potential for selection of tetraploid and diploid parental fish and shellfish with improved economic traits which can be crossed to produce triploids with improved traits of interest."*

Acceptance of the triploids groupers and salmons in aquaculture

Interspecies hybridization is widely used in aquaculture as a beneficial strategy. Diploid and triploid hybrids have been detected from the interspecies hybridization of Epinephelus coioides(female) X Epinephelus lanceolatus (male). Measurements of erythrocyte nuclei indicate that triploid fish have a larger nuclear surface than the diploid groupers, and the average ratio of triploid to diploid surface area is 1.59. The triploid hybrids had been proved to possess superior growth performance to that of the diploid hybrids and the parent species (Huang et al., 2014). During the first 1.5 years, triploid hybrid groupers grow faster than diploid hybrid groupers or either parent species. The average growth rate of triploid hybrids is 1.61 times greater than that of diploid hybrids at 6 months of age and 1.43 times greater at 18 months of age. The triploid hybrid groupers are inferior in gonadal development, with no primary-growth-stage oocytes appearing in the gonads at 18 months of age (Huang et al., 2014). To date, applications of polyploidy in grouper has not been well documented or explored. (Li et al., 2014b)

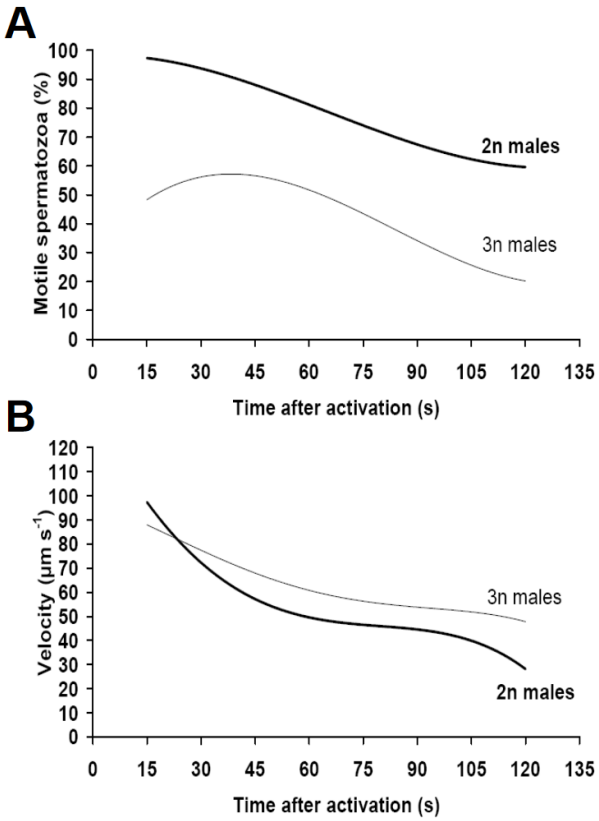

Figure 14: Sperm motility and velocity in the atlantic salmon for the triploids (3N) vs the diploids (2N)

On the other hand, during the first introduction of triploid Altantic salmon to the farmers, many fish farmers believed that triploid animals are more prone to diseases than diploids, but there is no published scientific evidence to support this. Research on complement activity in triploid Atlantic salmon suggests that they may be slightly disadvantaged compared to diploids in their ability to withstand exposure to bacterial pathogens (Langston et al., 2001). The only published study of disease resistance in triploid Atlantic salmon found them to be no different from diploids in their susceptibility to bacterial kidney disease, but this study used triploids and diploids from different families (Bruno and Johnstone, 1990). Studies with other salmonids have generally found no effect of triploidy on complement and phagocyte activity, vaccine efficacy or disease resistance (Benfey, 1999; Maxime, 2008), although Jhingan et al. (2003) did find triploid coho salmon (Oncorhynchus kisutch) to be less resistant than diploids to vibriosis from Vibrio anguillarum. Similarly to the groupers and most of triploids, the gonads of the triploid salmon are atrophied. Credits of photo in next page to (Felip et al., 2001) (Piferrer et al., 2009c)

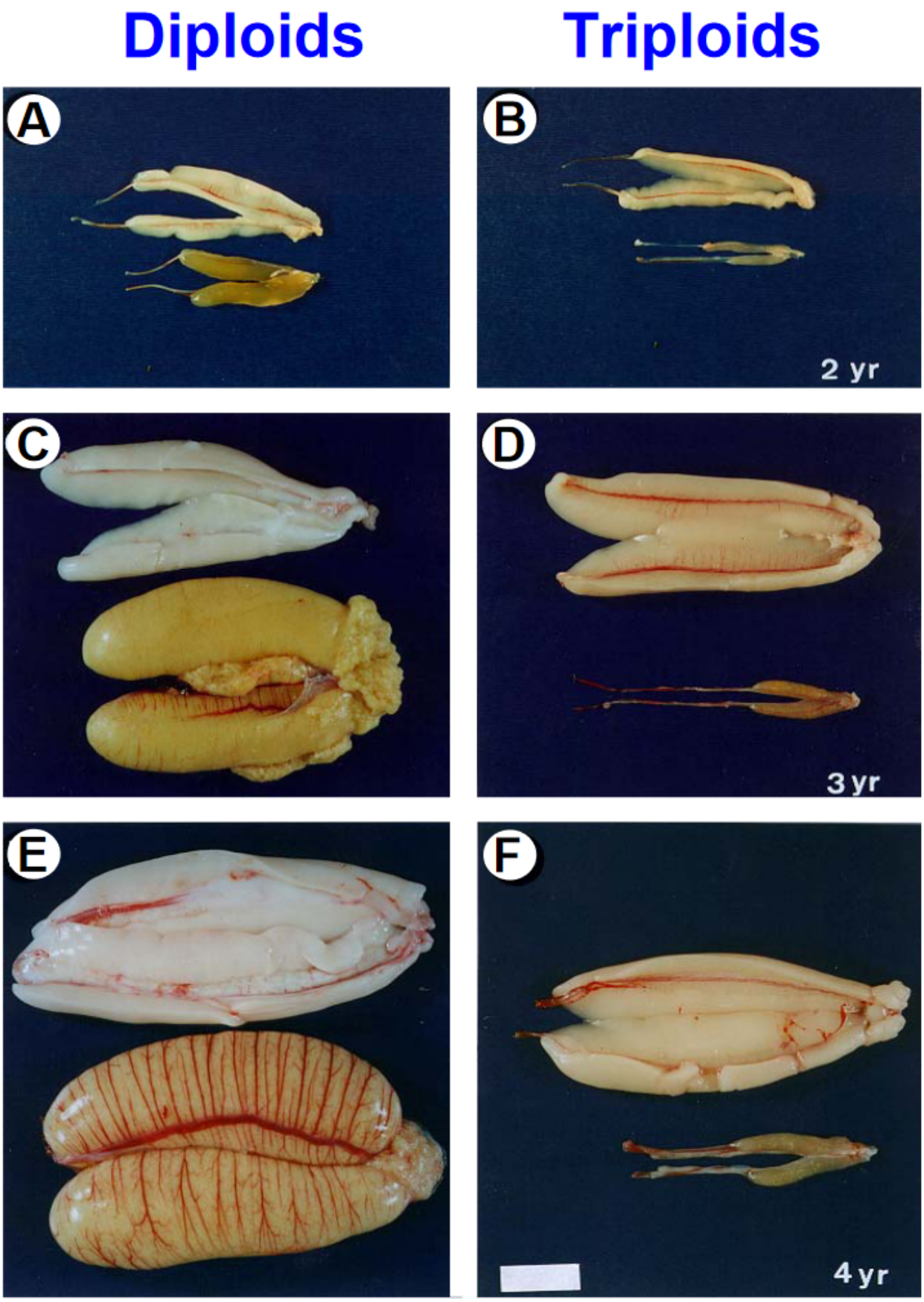

Figure 15: Comparisons of the gonads of a diploid salmon (normal gonads) versus a triploid salmon (atrophied gonads)

Advantages and Disadvantages

Polyploids, once passed the bottleneck of instability and viable, can possess 3 obvious advantages: heterosis, gene redundancy and unisexual reproduction that might fuel evolutionary success and enrich species diversification. Poly-ploidization may lead to instantaneous speciation. (Coyne and Orr, 2004)

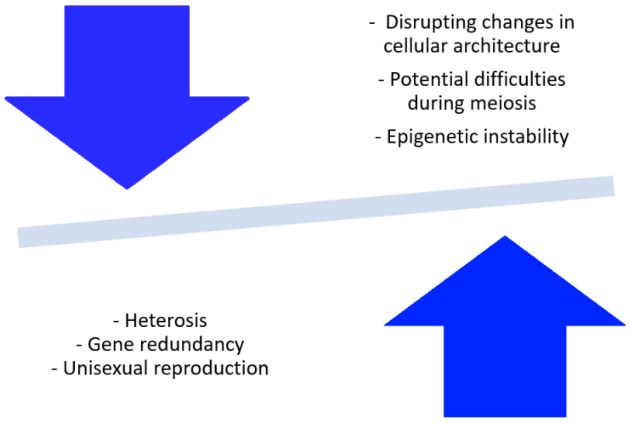

Figure 16: Main advantages and disadvantages of the triploid state in grouper

Polyploid fish such as triploids gain a clear advantage over diploid fish in general. This advantage is known as heterosis vigor. The heterozygosity divergence of duplicate genes mitigate the inbreeding pressure by increasing the expression of key physiological proteins. While polyploid fish do not differ considerably from diploids phenotypically, their fitness vary upon the individual and species.

Advantages

First, the increased number of alleles of a given gene in a polyploid should allow the masking of deleterious recessive mutations and thus insure against the loss off fitness.

The second proposed advantage of allopolyploids and heterozygous autopolyploids is that heterosis allows offspring to display transgressive performance compared with its progenitor species. In contrast to diploidhybrids where hybrid vigor decays over subsequent generations, heterosis is stable in allopolyploids, the explanation is in the dominant disomic pairing of identical homologous chromosomes. (Comai, 2005; Ohno, 1970)

The third major advantage is the neo-functionalization or sub-functionalization of the alleles issued from the duplicated genome, potentially allowing for ecological niche expansion or increased flexibility in the organism's responsiveness to the environmental change. (Adams and Wendel, 2005; Lynch, 2007; Moore and Purugganan, 2005)

Disadvantages

Possibility of less vigor and reduced adaptive capacity in polyploids due to the reverse effect of the first advantage: too much expression of deleterious alleles due to the increased number of chromosomes.

Difficulty in the reproduction: greater complexity of their pairing and segregation interactions would provoke defects such as aneuploidy during meiosis and mitosis.

Additionally ,the cell architecture in polyploids is altered because of generally increased cell size in polyploids, which alters the surface to volume-ratio(salmon cells are 50% larger in 3N) however the abundance of mRNA is balanced. (Levin, 2002; Melaragno et al., 1993; Rhoades and Dempsey, 1966)

Finally, changes in polyploids that can be either advantageous or detrimental are the altered transcriptome, genomic architectureand epigenetic landscape, which can lead to gene silencing or activation, as well as DNA loss and epigenetic changes (Hegarty et al., 2006; Wang et al., 2004; Rapp et al., 2009)

Conclusion

The triploid technology is a strong tool for the control of fertility and is a great achievement from the aquaculture industry. Although the performances and traits are not always improved in triploid fish, the discovery is exceptional in term of management of the reproduction. Induction of triploidy but unfertile hybrids save the environment by limiting the risk of unwanted reproduction between a farmed animal and a wild one. In the end the technology will develop and could become a popular tool making aquaculture more sustainable and ecological especially large size offshore salmon farms.

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Appendix

Packagename	Version	Maintainer
pacman	0.5.1	Tyler Rinker <tyler.rinker@gmail.com>
ggalt	0.4.0	Bob Rudis <bob@rud.is>
hagr	0.0.0	Andrew B. Collier <andrew.b.collier@gmail.com>
randomcoloR	1.1.0.1	Ron Ammar <ron.ammar@gmail.com>
kableExtra	1.3.4	Hao Zhu <haozhu233@gmail.com>
plotrix	3.8.1	Jim Lemon <drjimlemon@gmail.com>
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tidyverse	1.3.1	Hadley Wickham <hadley@rstudio.com>

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