1. INTRODUCTION

Rainbow trout, *Onchorynchus mykiss* (Walbaum, 1792) one of the most exploited aquaculture species in the world, will serve as the model in this thesis. This study evaluates the development, growth and mineralization of the skeletal system of this species during 219 days post fertilization. The effect of two different treatments on skeletal formation are evaluated, i) triploidization, in which an extra set of chromosomes is retained and ii) administration of a diet poor in phosphorus for 71 days, after which a diet with adequate phosphorus content was offered in order to assess the capacity of the developing skeleton to recover from a low mineral diet. To study modifications in the whole body mineral homeostasis in diploid and triploid trout and evaluate the effect of these challenge on the musculoskeletal system.

The following introduction will present the main themes which will be addressed in the thesis. This will include brief considerations about vertebrate development, the musculoskeletal system considering formation and structure of the skeleton and muscle, homeostasis of bone and the importance of minerals in the process of skeletal ontogeny. There is also an overview on the genetic tools available and the use of polyploids.

1.1. Development

1.1.1. General overview

Developmental changes can be either morphological (eg. changes in shape or structure), biochemical (eg. synthesis of specialized proteins), or both. Developmental changes in an organism include all of the changes that occur during the life of an organism, and include fertilization, embryogenesis, maturation, and gametogenesis. Embryogenesis initiates with fertilization of the gametes to form the diploid zygote. The zygote undergoes cleavage and divisions to form the blastoderm embryo (figure 1.1 presents schematically the development of the vertebrate embryo). Changes in cell shape and cell movements at gastrulation lead to the creation of three germ layers: the ectoderm, endoderm, and mesoderm (Creighton, 1999).

1.1.2. Ectoderm, endoderm, and mesoderm

The three primary germ layers, the ectoderm, mesoderm and endoderm are established during gastrulation (figure 1.1 M). These major lineages are progressively patterned and specialized originating tissues and organs that build the embryonic body plan. The mesodermal germ layer originates a range of tissues and organs, including the embryonic tissues prechordal plate, notochord, somites, heart, pronephros, and hematopoietic precursors.

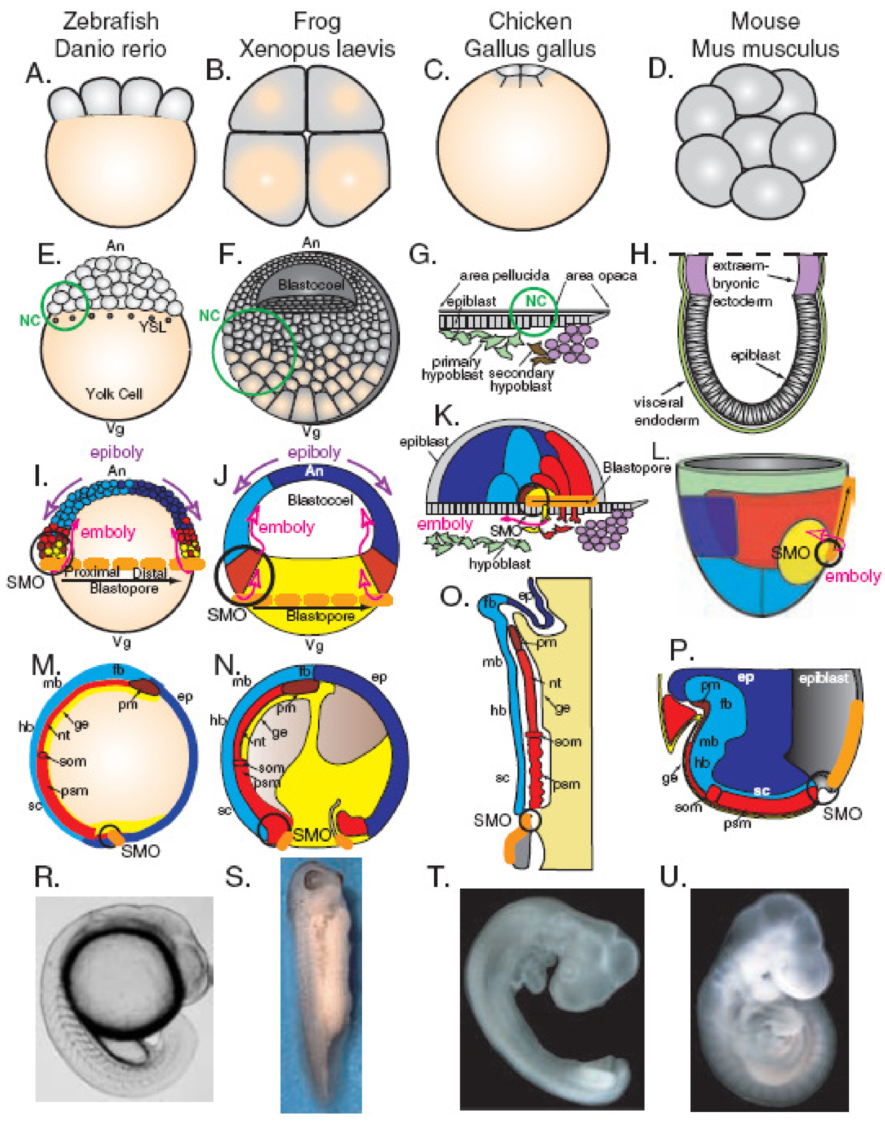

Figure 1.1 - Squematic representation of four vertebrate model organisms: zebrafish (*Danio rerio*), frog (*Xenopus laevis*), chick (*Gallus gallus*), and mouse (*Mus musculus*); comparing

embryos cleavage, blastula, and gastrula stages. Developmental stages of zebrafish (A, E, I, M, R), Cleavage, 8-cell stages (A through D). Note an incomplete cleavage in zebrafish (A), Early blastula (E through H), late blastula to early gastrula (I), late gastrula (M), and pharyngula (R). The position of the Nieuwkoop center (NC) and its equivalents is shown in the cleavage stages, and the position of the Spemann–Mangold organizer region (SMO) is shown in the early and late gastrula stages. The gastrulation movements of epiboly and emboly are illustrated during the early gastrula stages (I through L). Light gray, Cytoplasm; beige, yolk, dark gray; epiblast region of amniote embryos; red, mesoderm and its precursors; dark red, prechordal mesendoderm; yellow, definitive endoderm and its precursors; dark blue, epidermis; lighter blue, neuroectoderm, green, brown, and violet, various extraembryonic tissues; orange, blastopore. **ep,** Epidermis; **fb**, forebrain; **mb**, midbrain; **hb**, hindbrain; **sc**, spinal cord; **nt**, notochord; **pm**, prechordal mesendoderm; **som**, somite; **psm**, presomitic mesoderm; **ge**, gut endoderm (Solnica-Krezel, 2005).

The mesodermal lineage contributes to skeletal muscle, bone, heart, kidney, blood, dermis, connective tissue, much of the circulatory system, multiple digestive organs, excretory tract, mesenchyme, mesothelium, peritoneum, the reproductive system, and the urinary system in the adult (Schier and Talbot, 2005). The endoderm germ layer gives rise to many vital organs, including the liver, the pancreas, the thymus and the thyroid, and it forms the epithelial lining of the gastrointestinal and respiratory tracts (reviewed by Wells and Melton, 1999). Additionally, the endoderm itself acts as a source of signals that regulate the development of mesoderm and ectoderm organs such as the anterior central nervous system and the heart. The main derivatives of the ectoderm (the outer germ layer) are the central and the peripheral nervous systems, the epidermis, and the placodes (Moody, 2007).

1.2. Musculoskeletal System

1.2.1. Skeleton origin and function

The vertebrate skeleton is the product of cells from three distinct embryonic lineages. The craniofacial skeleton is derived from the cephalic paraxial mesoderm and invading cranial neural crest cells; the axial skeleton is derived from paraxial mesoderm in the vicinity of the notochord and neural tube; and the appendicular skeleton is the product of lateral plate mesodermal cells. The primordia of these tissues are specified early during ontogeny (Moody, 2007).

Undoubtedly, the most important function of skeleton is to maintain the structural integrity of the body for normal posture and locomotion. The skeleton provides an organized structure for muscle attachment and protects vital organs and cells and also serves as a reservoir for ions. The skeleton, like every system, has specific developmental and functional characteristics that define its identity (reviewed by Lall and Lewis-Mc Crea*,* 2007; Karsenty, 1999).

The vertebrate skeleton has evolved specific adaptations which are associated with species, life stage and the environment. For sharks and rays the cartilaginous skeleton is the terminal stage, and all of the essential components of the skeleton are already present at this evolutionary stage. In bony fishes, the skeleton is generally avascular although mineralization occurs. The skeleton developed in air-breathing vertebrates is lightweight and vascular. In terrestrial vertebrates, cartilage is mostly replaced by a type of hollow, vascular bone (reviewed by Blair *et al*, 2008)**.**

1.2.2. Skeletal tissue metabolism

In most vertebrates the skeleton serves as a reservoir of minerals such as, calcium (Ca) and phosphorus (P) which are continually exchanged with the electrolytes found in blood and extracellular fluid. Thus, the skeleton of most vertebrates acts as buffer on changes in plasma electrolyte levels (Blair *et al,* 2008). Biochemical mechanisms involved in skeletal tissue metabolism in fish are less well characterized and probably differ substantially from mammals. Unlike terrestrial animals, bone is not considered a major site of Ca regulation in fish (reviewed by Lall, 2002) and the regulation of Ca influx and efflux occurs in the branchial epithelium, intestine, fins and oral epithelia (Vielma and Lall, 1998; Guerreiro *et al,* 2007). For this reason in fish dietary phosphorus (P) supply is essential and may be a limiting factor for bone mineralization. Inorganic phosphorus is essential for a wide range of processes; it is an integral component of nucleic acids, a substrate for enzymes, important in intracellular signaling and for energy provision through ATP. Moreover, phosphorus is important in all tissue (eg. phospholipids membrane) and accumulates not only in the skeleton but also in soft tissue such as, heart, liver, kidney, muscle and blood (Lall and Lewis-McCrea, 2007; Guerreiro *et al,* 2007).

The fish skeleton is a complex metabolically active tissue that undergoes continuous remodeling. Morphologically, fish bones consist of the head bones, internal skeleton, and scales. However, fish do not have any hematopoietic elements (like mouse, human or frog) and the development of the skeleton in bony fish (Teleostei) follows a pattern that has been conserved during vertebrate evolution but specialized in bony fish where hematopoiesis occurs in the kidney (Huysseune, 2000; Witten *et al*, 2004; Moody, 2007).

1.2.3. Skeletal tissue composition

Physiological functions of bone tissue are carried out by three main type of cells: osteoblasts (bone forming cells which are originated at mesenchyme and secrete non-mineralized bone matrix - osteoid), osteocytes (entrapped inside the bone matrix and responsible for maintenance of bone substances and the exchange of ions, osteocytes connect to other osteocytes and to osteoblasts on the bone surface via long cell processes), and osteoclasts (multinucleated bone resorbing cells). Osteoblasts synthesize proteins of the extracellular bone matrix, promote calcification, and are involved in induction or downregulation of osteoclasts. Molecular markers present in early stages of osteoblast differentiation in mammals are type I collagen and alkaline phosphatase. In later stages of osteoblast differenciation is typical osteocalcin and mineralization of the extracellular matrix. Osteoblast markers include osteonectin, osteopontin, and bone sialoprotein (table 1.1 summarizes bone components) (reviewed by Cohen 2006, Witten *et al*, 2004).

Table 1.1 – Components of mammalian bone (Adapted from Cohen, 2006).

Components of Bone		
Cells	**Mineralized matrix**	**Organic matrix**
	Calcium Hydroxyapatite	
	Carbonate	Type I collagen (88%)
Osteoblasts	Citrate	Other proteins (10%)
	Fluoride	Osteocalcin
Osteocytes	Chloride	Osteonectin
	Sodium	Phosphoproteins
Osteoclasts	Magnesium	Lipids
	Potassium	Glycosaminoglycans (2%)
	Strontium	

Osteoclasts are only found in bone and are responsible for bone resorption, causes its breakdown and these cells are important in bone development when remodeling is required. The interplay between osteoclast and osteoblasts is important in bone homeostasis and ensures the breakdown and *de novo* bone formation is balanced (reviewed by Karsenty, 1999; Boyle *et al,* 2003). In humans if bone homeostasis is disturbed it can lead to disease such as osteopenia, osteoporosis, or Paget's disease, and too little can lead to osteopetrosis. Bone remodeling not only repairs and renews skeletal structures, but it it also contributes to homeostasis of serum calcium and phosphorus. These level of complexity is dependent on the communication with the gut, kidney, thyroid (parathyroid) and, in the case of fish, with the gills and the corpuscles of Stannius (Boyle, 2003).

Mammalian bone, fish bone and scales have a similar composition. The tissue is composed of calcium hydroxyapatite salts embedded in a matrix of type I collagen fibers. Thus essential supportive tissues are comprised of an organic bone matrix and inorganic minerals. The organic bone matrix is mostly composed of collagen and hydroxyapatite, a hydroxylated polymer of calcium phosphate [$Ca_{10}(PO_4)_6(OH)_2$] (Moss, 1961; Simkiss and Wilbur, 1989) while cartilage consists of cells in an extracellular matrix, which may or may not be mineralized depending on the cartilage type (Cartilage is classified in three types, elastic cartilage, hyaline cartilage and fibrocartilage, which differs in the relative amounts of the extracellular matrix content) (Hall, 2005).

In vertebrates, cartilage is a type of dense connective tissue that primarily consists of glycosaminoglycans, mainly chondroitin sulphates and proteoglycans. Cartilage structure and composition is variable and tissues with histological characteristics between bone and cartilage (eg. chondroid bone) have been identified in fish, and play an important role in skeletal development. Scales do not contain enclosed cells, but the cells important for their formation and turnover are in close apposition (reviewed by Lall and Lewis- McRea, 2007). In the largest group of vertebrates – teleosts (considered advanced teleosts), many of their osseous elements, the main cellular components of bone, the osteocytes, are missing. This phenomenon was first detected by Kölliker in 1859, about 150 years ago and since then has been described in detail in several species. The osteocyte-lacking bone of advanced teleosts is called anosteocytic or acellular bone, in contrast to the osteocytic or cellular bone of tetrapods, basal teleosts, and primitive osteichthyans (Moss, 1961; Meunier, 1987; Meunier and Huysseune, 1992).

1.2.4. Skeletal cell differentiation

Skeletal bone formation (ossification) can occurs by two distinct mechanisms, which are endochondral ossification (developing by the replacement of a cartilagineous model) or intramenbranous ossification (developing by the replacement of a fibrous or fibrocellular model). In intramenbranous ossification process skeletal elements, mesenchymal cells differentiate directly into osteoblasts. The process of bone formation called endochondral ossification is a result of mesenchymal cells differentiation into chondrocytes. The cartilaginous template is then replaced by bone containing osteocytes and osteoclasts, with exception for the acellular bone which has been regarded as ‘dead’ bone although osteoclasts do invade it. (reviewed by Blair et al, 2008; Karsenty and Wagner, 2002)

1.2.5. Skeletal tissue endocrine regulation

In tetrapods, the endocrine factors that control calcium (Ca^{2+}) and ionic phosphate (Pi) are well characterized and include parathyroid hormone (PTH), calcitonin, 1,25-dihydroxy vitamin D and FGF23 (Guerreiro *et al* 2007; Quarles, 2008). One of the most important endocrine regulators of Ca^{2+} and Pi external fluid concentrations is PTH. If calcium levels decrease in blood, PTH is secreted until normocalcemia, this effect is counteracted by calcitonin. The normalization of plasma calcium by PTH is accomplished by *1*) inducing the differentiation and activation of osteoclasts that mobilize Ca^{2+} and Pi from bone, *2*) stimulating the production of 1,25-dihydroxyvitamin D_3 from the small intestine, and *3*) stimulating tubular reabsorption of Ca^{2+} in the kidney, and reducing Ca^{2+} loss in urine. Also in parallel, PTH acts to decrease the kidneys Pi reabsorptive capacity. Thus, whereas Pi absorption from the intestine is efficient and minimally regulated, the concentration of Pi in the blood is reduced due to a PTH-stimulated loss in urine (reviewed by Guerreiro *et al,* 2007).

A hormonal cascade involving FGF23 and Klotho (gene that encodes a transmembranar protein) has been identified it principally regulates phosphate (Pi), vitamin D homeostasis, and mineralization of bone. FGF23, which is produced in the osteocytes, is the principal phosphaturic hormone in mammals and may function to counteract the hypercalcemic and hyperphosphatemic effects of excess 1,25 $(OH)_2D_3$ through reductions in PTH and elevations in FGF23 levels. The bone functions as an endocrine organ producing FGF23 and participates in a bone-kidney axis, regulating phosphate, vitamin D, and mineral homeostasis (Quarles, 2008).

The pituitary endocrine axis is also a bone formation stimulator in mammals via the growth hormone/IGF-1 axis, which regulates bone resorption (positively and negatively) via follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). Hormones from the anterior pituitary, growth hormone, TSH and FSH, also regulate bone mass. Growth hormone is anabolic, but exerts its effects via insulin-like growth factor-1 (IGF-1) from the liver. Genetic IGF-1 deficiency causes profound growth retardation and osteopenia (reviewed by Blair *et al,* 2008). There are major differences in the hormonal mechanisms that regulate Ca and P balance in fish and terrestrial animals. The endocrine control of Ca and P bone metabolism is regulated by hyper- and hypocalcemic hormones and not by parathyroid hormones (PTH). In fish stanniocalcin is the predominant hormone that regulates Ca and P homeostasis in freshwater, seawater salmon and several freshwater fish. Moreover, estrogen appears to be associated with bone resorption in fish possibly through the action of the recently identified PTHrP. The possible roles of PTHrP in fish skeletal development remain unclear, further studies need to be done. (Guerreiro *et al*, 2007; Fuentes *et al*, 2006; Abbink *et al*,2006). Fish are surrounded by an abundant supply of calcium in water, though it is unlikely to be a limiting factor, in contrast phosphorus can only be attained through the diet and this factor is probably of primary importance (reviewed by Lall and Lewis-McCrea, 2007).

1.3. Skeletal Muscles

1.3.1. General

Skeletal muscle in vertebrates is derived from mesoderm which forms segmented somites in the trunk (figure 1.2) and matures in an anterior to posterior direction (reviewed by Buonanno and Rosenthal, 1996). The process of somite development in zebrafish is similar to that in amphibians, birds and mammals. In zebrafish, at the end of the first day of development, somite formation is complete and somite patterning is nearly so (figure 1.2) (reviewed by Stickney *et al*, 2000).

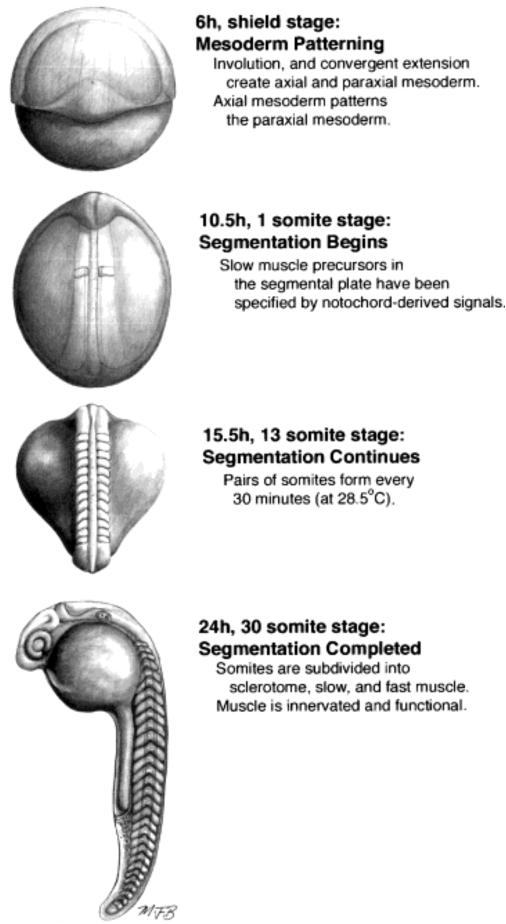

Figure 1.2 - Overview of zebrafish embryogenesis. These drawings show the stages of embryogenesis during which segmentation and patterning of the paraxial mesoderm takes place. The paraxial mesoderm develops from cells around the margin of the early gastrula, converging toward the dorsal side, forming paraxial mesoderm adjacent to the axial mesoderm that is derived from the shield. This convergence of cells toward the future notochord contributes to the anteroposterior extension of the embryo. The first somite forms shortly after the end of gastrulation. As somitogenesis continues, the trunk begins to lift off of the yolk and the tail extends. At the end of the first day, somite formation is complete and somite patterning is almost finished (Stickney *et al,* 2000).

In vertebrates muscle growth occurs by hyperplasia, formation of new muscle fibres, and volumetric growth of these fibers by hypertrophy. In most vertebrates, hyperplasia is limited to early stages of development, and postnatal growth occurs only by hypertrophy. One exception to this rule is fish in which muscle recruitment continues throughout the life cycle and for this reason fish grows indeterminately and size is never fixed (reviewed by Mommsen, 2001).

The formation of muscular tissue, myogenesis, the fundamental events common to all vertebrates are the specification of stem cells to a myogenic lineage (myoblasts), proliferation, cell cycle exit, differentiation, migration and fusion. (reviewed by Johnston, 2006).

1.3.2. Organization of muscle structure

In fish larvae, adaptation to a hydrodynamic environment and the lack of functional gills require the development of two special larval muscle areas: an inner white muscle zone and a superficial red layer. At hatching, the level of differentiation of these zones varies between fish species, these zones are present when the yolk sac is depleted and the larva has to uptake food and find a new source of energy to survive allowing further muscle growth.

In most fish, slow and fast muscle fibers occupy distinct regions of the body. Fast muscle fibers (also known as white muscle - anaerobic), comprise the deep portion of the myotome, which makes up most of the trunk musculature. Slow muscle fibers (or red muscle-aerobic fibers) are segregated into a wedge shaped region of the myotome at the lateral end of the horizontal myoseptum, which separates the hypaxial and epaxial muscle. Intermediate muscle fibers (pink muscle) are located between the slow and fast muscle fibers, within the deep region. Usually in most vertebrates, slow muscle fibers are small, darkly colored, more heavily vascularized, and contain more lipid and mitochondria than the large, pale fast muscle fibers. A fascia separates the slow muscle fibers from the intermediate and fast muscle fibers (reviewed by Koumans *et al,* 1995; Devoto *et al,* 1996).

1.4. Mineral requirement

1.4.1. Calcium and phosphorus

Calcium and phosphorus are closely related to the development and maintenance of the skeletal system and the stability of the vertebra depends on calcium phosphate. Fish and other aquatic organisms absorb Ca and P from water and their Ca requirement is met by their ability to absorb this element directly from water. However, the concentration of P is low in both freshwater and seawater and diet is the main source.

The absorbed calcium is deposited in bone, scale and skin. The chemical composition of the bony layer of scales is similar to that of other skeletal tissues but differs physiologically in Ca metabolism. The scales are also a site of labile Ca storage. The regulation of phosphate is considered more critical than that of Ca because fish must effectively absorb, store, mobilize, and conserve phosphorus in both freshwater and seawater environments (reviewed by Lall, 2002).

1.4.2. Phosphorus deficiency deformities

Calcium deficiency is considered to be uncommon in fish because of their ability to take up Ca from water through their gills and intestine. The amount of circulating Ca^{2+} is maintained under strict control, because slight changes may disturb a range of physiological mechanisms including neural, muscular, and cardiovascular functions, leading to tetany, lethargy, and, ultimately, death. Deficiency of P can occur more easily, and signs of deficiency include reduced growth, decreased feed efficiency, reduced bone mineralization and skeletal abnormalities (reviewed by Lall, 2002; Guerreiro *et al,* 2007). Common skeletal deformities include curved spines and soft bones in Atlantic salmon, cephalic deformities in the frontal bones in common carp, and compressed vertebral bodies resulting in scoliosis in haddock and halibut (Lewis-McCrea and Lall, unpublished results). Atlantic halibut and haddock juveniles fed low P diets have twisted neural and hemal spines in the mid section of the fish (Lewis-McCrea and Lall, unpublished results) which has been suggested to be the consequence of the action of muscle contraction on the poorly calcified bone.

Histological and histochemical examination of P deficient haddock showed an initial increase in bone resorption, which was subsequently followed by a decrease in bone mineralization and reduced bone formation. These observations are based on the increase in the number of osteoclasts and suggest that osteoclasts may be involved in P homeostasis. In hypophosphatemic fish exhibiting bone deformities, a deceleration of bone mineralization occurs due to an increase in the amount of osteoid tissue and number of osteoclasts and a decrease in the number of osteoblasts (reviewed by Lall and Lewis-McCrea, 2007).

1.5. Biotecnology applied to aquaculture and lifesciences

Biotechnology provides tools to transform and develop biological systems, for production of high quality fish (reviewed by Maclean and Laight, 2000). As in many other research areas of life sciences, modern biotechnology is considered to be a new tool to improve the quality and quantity of fish reared in aquaculture. Finfish and shellfish can be genetically modified through gene transfer; chromosome set manipulation, interspecific hybridization, and other methods. Biotechnology is, however, not only designed for the creation of broodfish with a superior genetic background and other applications can be valuable detection tool for the prevention, control and management of various diseases and also for genetic selection of traits of commercial interest. Genetically modified organisms and general biofarming is another emerging field of research that exploits biotechnology, and for example, tilapia are being used for the production of insulin (Beardmore and Porter, 2003). Aquatic animals attract more research attention than terrestrial livestock. Fish lay eggs in large quantities and these eggs are easily manipulated, making it easy to insert novel DNA and development occurs independent of the broodstock. Cows and pigs produce fewer eggs at a time, and once novel DNA is inserted, manipulated eggs must be re-inserted into the animal (reviewed by Aerni, 2004).

1.5.1. Ploidy vs Transgenics

A diploid cell has two copies of each chromosome, one from the mother and one from the father. Cells with more than the normal complement of DNA in their genomes, usually with increased numbers of the standard chromosomes, are called polyploid cells. There are polyploid forms called triploid (three genomes), tetraploid (four genomes), pentaploid (five genomes), and so on. Each of the chromosomes is repeated by the same integer. During meiosis, polyploidy is maintained in subsequent generations if there is an even number of genomes (reviewed by Creighton, 1999; Rasmussen and Morrissey, 2007).

Triploidy is the condition in which somatic cells contain three sets of chromosomes. It is widespread in plants and occurs sporadically in lower vertebrates (fishes, amphibians and reptiles). In fishes, autotriploidy (within species) and allotriploidy (between species) are both caused by disturbances in the ripening mechanisms of the eggs with a subsequent blocking of the extrusion of the second polar body, represented in figure 3 (reviewed by Maxime, 2008).

The first transgenic animal to be produced was a mouse (Palmiter, Brinster and Hammer, 1982), which was manipulate their growth rate with additional growth hormone (GH) genes. The first recorded instances of production of transgenics in aquatic species are those of Maclean and Talwar (1984) in rainbow trout and Zhu *et al.* (1985) in goldfish (Melamed et al., 2002). The process of genetic modification is a multistage process which can be summarized as: 1. identification of the gene interest; 2. isolation of the gene of interest; 3. amplifying the gene to produce many copies; 4. associating the gene with an appropriate promoter and poly A sequence and insertion into plasmids; 5. multiplying the plasmid in bacteria and recovering the cloned construct for injection; 6. transference of the construct into the recipient tissue, usually fertilized eggs; 7. integration of gene into recipient genome; and 8. expression of gene in recipient genome; inheritance of gene through further generations (reviewed by Beardmore and Porter, 2003).

In our days a variety of transgenic species are being engineered for aquacultural, industrial, and pharmaceutical applications. The main goals in the transgenic research are the growth enhancement and disease control (like cold disease tolerance, vaccines). The efficiency of growth and feed conversion is increased in finfish by creating transgenic fish that incorporate a gene construct encoding growth hormone, resulting in a 3–11-fold gain in weight. Although overexpression of GH in transgenic fish can result in pleiotropic effects in addition to enhancing growth rates. In some cases, expression can be at a level which results in reduced viability and morphological abnormalities resembling acromegaly. Effects on disease resistance, metabolism, endocrinology, swimming ability, organ structure, and behaviour have also been observed in several species. Transgenesis provided a useful tool for developing model fish systems for examining the effects of GH on many physiological processes (Devlin et al., 2004).

The taxionomic group with the most transgenic species, are aquatic organisms, which are also one of the groups most likely to present environmental concerns if accidentally released into natural ecosystems. Unlike most other agricultural species, fish are both difficult to contain and highly mobile, and they can easily become feral and invade native ecosystems (Wong et al., 2008).

1.5.2. Triploid fish production

Triploid fishes can be artificially produced and this method has been applied to a vast number of species. The first trials of ploidy manipulation on fish chromosomes were carried out in the 40's, then Swarup in late 50's was the first to successfully rear triploid fishes to adulthood and to compare their growth and sexual maturation with those of diploid controls. The main interest in triploidyzation is to produce bigger animals without having the problems associated with sexual maturation, such as, decreased growth of females, lipid mobilization, alteration in pigmentation, and ultimately texture detioration. The production of triploids is possible using several techniques (see table 1.2), although heat treatment (or heat shock) and the hyperbaric treatment (or pressure) are the methods most frequently used (reviewed by Maxime, 2008; Kacem *et al,* 2003; Swarup 1959).

Table 1.2 – Methods applied for inducing triploidy in fish (Adapted from Tiwary *et al,* 2005).

Method	**Species**	**References**
Cold Shock	*Oncorhynchus mykiss*	Thorgaard and Gall (1979)
Heat Shock	*Oreochromis mossambicus*	Varadaraj and Pandian (1988)
Pressure Shock	*Danio rerio*	Streisinger *et al*. (1981)
Nitric Oxide	*Salmo salar*	Johnstone at al. (1989)
Freon	*Salmo salar*	Johnstone *et al*. (1989)
High pH & Ca^{++}	*Oncorhynchus mykiss*	Ueda *et al*. (1988)
Cytochalasin B	*Salmo salar*	Retsti *et al*. (1977)
Colchicine	*Salvelinus fontinalis*	Smith and Lemoine (1979)

Triploids are produced by preventing the egg undergoing meiosis so that it remains in the diploid (2n) state. When the egg is fertilized by sperm in the 1n (haploid) stage the result is a triploid embryo (Figure 1.3).

As stated earlier triploid nuclei contain an extra set of chromosomes, so their DNA content is 1.5 times greater than that of diploid nuclei. However, Suresh & Sheehan (1998) found that the white muscle tissue of triploid and diploid rainbow trout contained more or less the same amount of DNA at a given body size. Several studies have shown that the number of cells and/or nuclei constituting a given tissue is reduced in triploid organisms, thereby causing the DNA content in triploids to be approximately the same as in diploids. It is not known whether the reduction in cell number in triploids modifies their physiological and functional (Suresh and Sheehan, 1998).

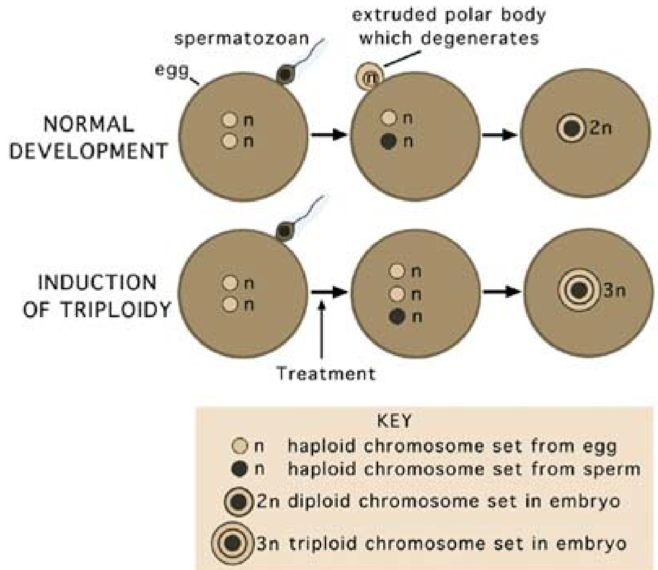

Figure 1.3 – General representation of the process of triploidy induction (Beardmore and Poter, 2003).

1.5.3. Morpho-anatomical changes in triploids

Morphological differences between diploid and autotriploid fish are not visible in most species of fish. The physiology of triploid fishes seems, in many aspects, to be very similar to that of the diploid fish despite basic biological differences (cytology, reproduction). Remarkable morphological similarities between triploids and diploids have been observed in rainbow trout. Lower jaw deformity in triploid Atlantic salmon is the most frequently reported skeletal abnormality associated with triploidy in fish. Tave (1993) observed deformities in the head of triploid bighead carp and grass carp. In contrast, Fast *et al* (1995) reported a reduction in frequency of deformities in triploid Asian catfish. Only two anatomical differences were reported between triploid and diploid fish in addition to impaired gonadal development. The first difference was the divided spleen in some triploid rainbow trout. The second difference is associated with muscle growth and development and Atlantic salmon displayed a lower density of satellite cells, reduced rates of fibre recruitment, hypertrophy of muscle fibres, advanced development of myotubes, myofibrils and acetylcholinesterase staining at the myosepta**.** A profound change in size of the air sac and spinal deformities were observed in triploid Indian catfish (reviewed by Tiwary *et al,* 2005).

1.5.4. Growth performances in triploids

Growth rate from a commercial point of view is an important characteristic for the genetic improvement in fish rearing. Since triploids are sterile or have reduced gonadal development it is expected that triploids have a higher growth potential. Although what is observed is that growth performances between diploids and triploids varies between species. Triploids in species such as rainbow trout, sunshine bass (a hybrid), coho salmon and Atlantic salmon tend to grow poorly when compared to their diploid counterparts (reviewed by Tiwary *et al*, 2005; Maxime, 2008).

Despite the great similarities between diploid and triploid fish (except cytological and reproduction difference), when these animals are subject to stress factors (such as suboptimal rearing and/or environmental conditions) there is a tendency for the triploid performance to be more severely affected than the diploid fish (reviewed by Maxime, 2008).

Investigations in triploid fishes have been mainly justified by economic considerations, although these studies have also led to some interesting observations about the cytological characteristics of triploids such as: hypertrophy of muscle fibres, high potential of tissue regeneration, lower tumour incidence, decreased environmental perception and consequences of cellular shape (reviewed by Maxime, 2008).

Triploids organisms represent an interesting resource for fundamental research. For example, the different cytology of triploids in relation to diploids, the modification in gene expression of transmembrane proteins may both be expected to cause fundamental modifications in physiological mechanisms and make the triploid an interesting alternative model (reviewed by Maxime, 2008).

1.6. The experimental model - Rainbow trout

The typical appearance of rainbow trout (*Onchorynchus mykiss*) is shown in figure 1.4. Rainbow trout is one of the first fish species to be domesticated and reared for human consumption and has been exploited for several hundred years.

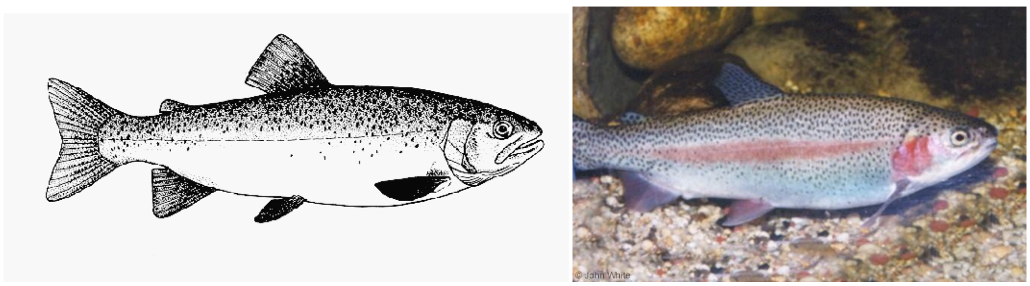

Figure 1.4 – Schematic representation and photograph of *Onchorynchus mykiss* (*Adapted from* Eccles, 1992). Picture from *John White* in http://animalpicturesarchive.com.

The species tolerates a considerable ranges of temperature variation (0-27°C), but spawning and growth occurs in a narrower range (9-14°C). The optimum water temperature for rainbow trout culture is below 21°C. As a result, temperature and food availability influence growth and maturation, causing age at maturity to vary, although it usually takes 3-4 years. Females are able to produce up to 2000 eggs/kg of body weight. Eggs are relatively large in diameter (3-7 mm). Most fish only spawn once a year in spring (January-May), although selective breeding and photoperiod adjustment has developed hatchery strains that can mature earlier and spawn all year round. The rainbow trout is fast growing, tolerant to a wide range of environments and handling, easy to reproduce and the large fry can be easily weaned on to an artificial diet (usually feed on zooplankton). In addition, because of the commercial importance of the rainbow trout means a large number of molecular resources have been developed and this facilitates molecular studies and favors it as a choice as experimental model (Cowx, 2005).