Anatomical features for the adequate choice of experimental animal models in biomedicine: I. Fishes

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ABSTRACT

Fish constitute the oldest and most diverse class of vertebrates, and are widely used in basic research because of a number of advantages (e.g., rapid development ex-utero, large-scale genetic screening of human disease). They represent excellent experimental models, to address studies on development, morphology, physiology and behaviour function in other related species, and also informative analysis of the conservation and diversity. Although less complex, fish share many anatomical and physiological features with mammals, including humans, which make them an important complement to research in mammalian models.

In this review we describe and compare the most relevant anatomical features of the most used teleostean species in research, to be taken into consideration when selecting an animal model: zebrafish (*Danio rerio*), medaka (*Oryzias latypes*), the turquoise killifish (*Nothobranchius furzeri*), and goldfish (*Carassius auratus*).

Zebrafish and medaka are the mainstream models for genetic manipulability and studies on developmental biology; the turquoise killifish is an excellent model for aging research; goldfish has been largely employed for neuroendocrine studies.

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Introduction

Basic scientific research, defined as experimental investigative research to advance knowledge without a specifically envisaged or immediately practical application, has been put in contrast to applied research, which seeks specific solutions to targeted problems by applying known fundamental results. This dichotomy should be overcome, since the two types of research are inter-twined and interconnected, depending on each other. Both basic and applied researches rely upon the use of animal models.

In basic research, mammalian models, such as rodents, have been pre-eminent in modelling human physiological and disease processes, because of many anatomical, physiological and genomic similarities, other than specific features (Lossi et al., 2015). However, mammalian models could be disadvantageous in certain types of research. Specifically, the large-scale genetic screening of human disease or developmental biology could be hampered by murine long gestation period or by *in utero* gestation. The rapid development ex-utero, allowing phenotypic analysis of embryogenesis and organogenesis *in vivo*, the transparency of embryos and larvae, allowing the *in vivo* visualization of cell biological events, the short lifespan made fish powerful and increasingly popular models in basic research. Furthermore, Fish represent an unvaluable tool for comparative and evolutive approach and for studying various branches of biology, thanks to their evolutionary position relative to those of other vertebrates, and to their high adaptive capacities. Fish constitute the oldest and most diverse class of vertebrates, comprising around 48% of the known extant species in subphylum Vertebrata. Part of the wide variability they exhibit has been attributed to a whole genome duplication event (Sidow, 1996; Meyer and Schartl, 1999), which occurred at the base of the teleost radiation (Christoffels et al., 2004; Vandepoele et al., 2004). The duplication of a gene/genome led to subsequent gene loss, to sub-functionalization or to neo-functionalization of the paralogs generated in the duplication event. After the initial genome duplication, the genomes of different teleost lineages evolved independently. The independent evolution of duplicated genes and the resulting sub-functionalization in fish can be useful for obtaining results that are impossible to get for the corresponding (non-duplicated) homologue in mammals (Furutani-Seiki and Wittbrodt, 2004).

Despite obvious differences, as fellow vertebrates, fish share many anatomical and physiological characteristics with mammals, including humans, which make them an important complement to mammalian models of disease. As a consequence, fish are used as experimental models to address function in other, often distantly related species, for

informative analysis of the conservation as well as diversity of processes that regulate development, morphology, physiology and behaviour (Lieschke and Currie, 2007).

In addition, scientists have been using Fish as substitutes of mammalian models in biomedical research, also as consequence of the debate on the use of animal research and the increasing awareness of the importance of humane use of vertebrates during experimental procedures. Fish are excellent examples of application of the 3Rs principles (replacement, reduction and refinement), according to Russell and Burch (1959) in research. Although fish display phenotypes resembling those of mammals (Santoriello and Zon, 2012), they possess less complex anatomical and physiological features. Furthermore, the great opportunity of conducting experiments on larval stages, when non-neuronal organs such as the heart are well developed, but the central nervous system (CNS) remains relatively primitive, perfectly fits with the replacement concept. The advantage of the size and transparency of fish larvae to perform similar procedures as those performed in mammals represents a less invasive and more refined method.

This review discusses in detail the advantages of using fish as experimental model, although fish diverged from humans more than 400-million years ago. Specifically, we will describe commonalities to justify conducting research that is relevant to humans in these animals, focusing on the main and relevant anatomical features of some of the most used teleostean species in research, to be taken into consideration when selecting an animal model: zebrafish (*Danio rerio*), medaka (*Oryzias latypes*), the turquoise killifish (*Nothobranchius furzeri*), and goldfish (*Carassius auratus*). Zebrafish and medaka represent the mainstream models for genetic manipulability (Schartl, 2014) and studies on developmental biology; the turquoise killifish is an excellent model for aging (Cellerino et al., 2015); goldfish has been largely employed mainly for neuroendocrine studies (Popesku et al., 2008; Stacey et al., 2003). Turquoise killifish and medaka belong to sister groups, respectively Cyprinodontiformes and Beloniformes; zebrafish and goldfish, to the order Cypriniformes. In this review we first give a short morphological description of each species, and then we describe and compare the most relevant anatomical features of the four species.

Fish species

Nothobranchius furzeri, also known as turquoise killifish, is a small freshwater fish, belonging to the order Cyprinodontiformes. It shows marked sexual dimorphism and dichromatism, and has a short maximum natural lifespan (<12 months) due to annual

desiccation of the pools in which they live. Notably, this short lifespan is also retained in captivity, varying between 3 and 18 months (Cellerino et al., 2015). This peculiar characteristic makes this killifish especially attractive for aging studies. The life cycle is entirely adapted to the ephemeral and unpredictable conditions of their habitat. Fish hatch when the pool is filled with water, grow rapidly and become sexually mature within few weeks (Polačik et al., 2011). After reaching sexual maturity, they reproduce daily. When aged, at macroscopical level, animals display loss of color, mainly observed in males; loss of body mass, which is reflected by a decrease in weight; curvature of the dorsal spine (Genade et al., 2005) and deterioration of the fins, especially the caudal fin (Lucas-Sanchez et al., 2011). At microscopic level, progressive increases in granules of lipofuscin, galactosidase and fluoro-Jade B in organs such as the liver, different areas of the brain (Terzibasi et al., 2009), skin (Valenzano et al., 2006) and gills (Hsu and Chiu, 2009), related to the cellular and tissue deterioration associated with aging. Furthermore, species of genus *Nothobranchius* shows a high incidence of tumorigenesis, high frequency of the onset of degenerative disorders in kidney and in liver (such as steatosis), in the heart (fibrosis and aggregations of lymphocytes around myocardial fibres) and the gonads (atrophy and fibrosis) (Lucas-Sanchez et al., 2014).

Oryzias latipes, also known as the medaka Japanese rice fish, is a small (2-4 cm long) fish, one of the 27 species in the ricefish family Adrianichthyidae, in the order Beloniformes. It is closely related to other members of the superorder Acanthopterygii of ray-finned fish such as pufferfish (tetraodon and fugu), stickleback and killifish, while it is separated from zebrafish by 150 million years of divergent evolution. Native to Southeast Asia, the medaka lives in slow-moving streams, coastal tide pools and rice paddies. Medaka are hardy fish that do well in a range of temperatures and water salinities; in the wild they migrate between fresh and salt water areas, and are thus common along coastal regions, in aquaria they prefer at least slightly brackish water.

Danio rerio, commonly known as zebrafish, is a small freshwater shoaling cyprinid fish, originated from shallow ponds and standing water bodies, often connected to rice cultivation in India. Zebrafish have fusiform, laterally compressed bodies that reach an average length of 25 mm. They have centrally located eyes and thin elongate mandibles with a protrusive lower jaw that causes the mouth to point upwards. Like other cyprinids, zebrafish are stomachless and toothless. As a result, they rely on gill rakers to break up food. Zebrafish have several defining features including an incomplete lateral line, two pairs of barbels, and longitudinal stripes along the sides of their body. The degree of

sexual dimorphism in zebrafish is minimal, as males tend to have more yellow coloration and tend to have larger anal fins than females.

Carassius auratus, also known as goldfish, is a member of the freshwater family Cyprinidae. There are several subspecies of *C. auratus*, all indigenous to Asia. The best known subspecies is *Carassius auratus auratus*, the common domesticated goldfish. The common goldfish has two sets of paired fins - the pectoral fins and pelvic fins, and three single fins - the dorsal, caudal, and anal fin. They lack barbels on the upper jaw, and lack scales on the head. Goldfish have exceptionally large eyes and acute senses of smell and hearing. They have 27-31 scales along their lateral lines. Goldfish have (rather than true teeth) pharyngeal teeth in their throats, they use to crush food.

Genome

High-quality genome sequence and complete annotation of protein-coding genes of fish with identification of their human orthologues is essential to enhance the understanding of the detailed roles of specific genes in human diseases, both rare and common, and the use of fish as model in biomedical research.

Data on all animal genome sizes are available on "Animal Genome Size Database" (http://www.genomesize.com). Although there are no significant different genome sizes among mammalian species, the situation changes considerably in fish species. For example, *Takifugu rubripes* (Aparicio et al., 2002) and *Tetraodon nigroviridis* (Jaillon et al., 2004) possess almost equal size of genome (400Mb), whereas medaka has 2-folds genome size and zebrafish 4-folds genome size (Imai et al., 2007).

The genome of *N. furzeri* has been sequenced and assembled and will likely be released in the near future (Cellerino et al., 2015). The *N. furzeri* genome is 1.6–1.9 Gb in size and is characterized by a high repeat content (45%) (Reichwald et al., 2009). However, a comprehensive, annotated *N. furzeri* transcript catalogue and a first transcriptome-wide insight into *N. furzeri* aging, useful for functional studies of aging-related genes (Petzold et al., 2013), is now available.

The medaka genome sequence project has been successfully completed (Kasahara et al. 2007) and the medaka draft genome is accessible through a number of genome browsers (http://www.ensembl.org/Oryzias_latipes/Info/Index). The assembled genome of medaka includes 800 megabases. The small genome represents an advantage compare to zebrafish (Kasahara et al., 2007), for studies in developmental genetics, genomics and evolutionary biology.

Zebrafish genome has been sequenced (Howe et al., 2013). Detailed automatic and manual annotation provides evidence of more than 26,000 protein-coding genes and, compared to the human genome, shows that approximately 70% of human genes have at least one obvious zebrafish orthologue (Howe et al., 2013).

The today goldfish is the artificial breed of the wild goldfish *Carassius auratus auratus* from China. It shows diverse morphologies in body shape and coloration. Sofar, complete genome sequencing is not available, although numerous genes have been identified and studied at expression levels (Unniappan et al., 2002). Molecular genetic markers, such as random amplified polymorphic DNA (Suzuki et al., 2005); microsatellite DNA (Jorge et al., 2012); and partial sequences of mitochondrial DNA (Yamamoto et al., 2010; Cheng et al., 2012; Kalous et al., 2012) have been used for the identification of species, ploidy, clonal lineages, and phylogenetic relationships.

Embryonic development

The advantage of using fish as model in vertebrate embryogenesis is also attributable to the external development of fish embryos from transparent eggs. The development is characterized by synchronous divisions, and absence of the G1 and G2 phases, thus cells proceed directly from the S to M phase (Graham & Morgna, 1966).

However, the developmental strategy of fish species is closely related with their life cycle. In annual fishes, such as *N. furzeri,* one-cell stage occurs at approximately 2 hours post fertilization (hpf) (Hartmann and Englert, 2012). Cleavage lasts 75 min, and produces a typical teleost blastula (Iwamatsu, 2004) during the first day after fertilization. The gastrula stage begins at day 2, and epiboly is completed at day 3. During epiboly, blastomeres disperse and appear arranged in a striking pattern of near-uniform distribution. The dispersion lasts for 5 days, and embryos might enter the first developmental arrest (diapauses I) (Hartmann and Englert, 2012). After re-aggregation, the neural keel forms, and then somitogenesis and morphogenesis of the nervous system proceed until the embryo enters diapause II. At this stage, the number of somites is fixed, the heart is tubular and contractile, the major divisions of the encephalon are present and so are the optic cups with the lens. The duration of diapause II is highly variable, from 2 days to up to 3 years, depending on the temperature (Valenzano et al., 2011). After diapause II, the embryo proceeds to complete development. *N. furzeri* does not show diapause III under standard laboratory incubation conditions. Hatching is a critical process for survival, also because of a very hard chorion to digest (Cellerino et al., 2015). In laboratory conditions,

hatching can be induced by hypoxia (Levels et al., 1986). After hatching and under optimal laboratory conditions, *N. furzeri* juveniles are immediately able to feed actively and show rapid juvenile growth: the fastest maturation observed in a vertebrate with a typical duration of 3–4 weeks, and one recorded case of 18 days from hatching to sexual maturity (Blažek et al., 2013).

The embryonic development of medaka, under laboratory conditions, consists of 39 stages, with the latter corresponding to the hatching, and lasts about 9 days (Iwamatsu, 2004). The female medaka spawns between 20 and 40 eggs every day within a hour after the onset of light. Fertilized eggs undergo cleavage, which takes approximately 30 min at 28.8 °C, gastrulation starts after 8.5 hpf and the neural axis is visible after 15 hpf. The blastula period begins with a horizontal division in the central blastomeres, at 128 cell stage the YSL begins to appear, at the end of this period the blastoderm has flattened down capping the yolk sphere. The gastrula period begins at 20% of epiboly, rhythmic contractions of the yolk occur and can be efficiently blocked by the addition of n-heptanol to the medium without interfering with embryonic development (Rembold and Wittbrodt, 2004), thereby allowing extended observations and time-lapse video microscopy. This period ends when epiboly is 90%, with appearance of brain rudiment, Kupffer's vesicle and optic vesicle. At stage of 2 somites, during segmentation, the epiboly is completed and organogenesis occurs along the somitogenesis, lasting up to 140 hpf (Iwamatsu, 2004). Embryos hatch after 8-9 days at 28°C as fully developed juvenile fish, able to swimm and feed.

The embryonic development of zebrafish begins about 40 minutes after fertilization. The zygote period lasts up to 3-4 hpf, until the first cleavage occurs. After the first cleavage, blastomeres divide at about 15 minute intervals. During blastula period, lasting from about 2 to 5 hpf, cells continue to divide synchronously, the marginal cells collapse, and release their cytoplasm and nuclei together into the immediately adjoining cytoplasm of the yolk cell. Thus the yolk syncytial layer (YSL) arises as prominent feature with Nomarski optics, and important for staging. The YSL, an organ unique to teleosts, may be extraembryonic, making no direct contribution to the body of the embryo. At first, the YSL has the shape of a narrow ring around the blastodisc edge, but soon (within two division cycles) it spreads underneath the blastodisc, forming a complete internal syncytium, that persists throughout embryogenesis. In the late blastula, epiboly begins (Solnica-Krezel and Driever, 1994), as the thinning and spreading of both the YSL and the blastodisc over the yolk cell. During gastrula period, lasting about 5 to 10 hpf, epiboly continues, and in addition, the

morphogenetic cell movements of involution, convergence, and extension occur, producing the primary germ layers and the embryonic axis. The segmentation period, from 10 to 24 hpf, is characterized by morphogenetic movements, and development of somites. Furthermore, the rudiments of the primary organs become visible, the tail bud becomes more prominent and the embryo elongates. Somites appear sequentially in the trunk and tail, and provide the most useful staging index. Anterior somites develop first and posterior last. There are no transient somites in the zebrafish; the first somite forms the first definitive myotome and so on. The pharyngula period (24-48 hpf), the time of development when one can most readily compare the morphologies of embryos of diverse vertebrates, corresponds to the second of the three days of embryonic development. The embryo shows a well-developed notochord, and a newly completed set of somites that extend to the end of a long post-anal tail. The nervous system is hollow and expanded anteriorly. During the hatching period, from 48 to 72 hpf, depending also of the temperature, the embryo continues to grow at about the same rate as earlier. Morphogenesis of many of the organ rudiments is rather complete and slows down considerably, with some notable exceptions including the gut and its associated organs. However, these endodermal structures are difficult to visualize in the living embryo because of their deep positions. Much easier to see are the rapidly developing rudiments of the pectoral fins, the jaws, and the gills. After 120 hpf (day 5), temperature dependent (Strähle et al., 2012), zebrafish become capable of swimming and feeding on external feed sources.

Finally, with regards to the embryonic development of the common goldfish, fertilized eggs are thick and not transparent, because of yolk texture, softer and larger compared to zebrafish. At one-cell stage, perivitelline space appears and cytoplasm moves to animal pole to form the blastodisc (Tsai et al., 2013). The cleavage period starts 40 minutes postfertilization and lasts up to about 3 hpf, forming 3 blastomeres layers. Blastula stages embryos are classified into high, oblong, sphere, and dome stages (Tsai et al., 2013). In the high stage, blastodisc shows an elliptical shape. In the oblong stage, the border between blastodisc and yolk is smooth and the blastomere shape remains elliptical. In the sphere stage the shape becomes spherical or highly compressed pear-shape, and, finally, in the dome stage yolk cell doming toward animal pole as epiboly begins (Tsai et al., 2013). The gastrula period last up to 12 hpf and is characterized by thickness of brain rudiment, tail bud prominence. At this stage, epiboly is completed and embryos possess one to five somites. The somitogenesis continues during the segmentation stage, which completes at 22 hpf with 22 somites. The rate of somite appearance is approximately two

somites per hour in the goldfish. The pharingula period, lasting up to 44 hpf, is characterized by pigmentation in retina and skin, red blood cells on yolk, median fin fold with well extended actinotrichia, heart beat, pectoral fin bud appearance. Finally, in the hatching period, beginning 58 hpf and lasting up to 72 hpf, the pigmentation is completed, embryos display distinct yellow colored head and dorsal body. Moreover, at this stage there is a distinctive well-developed pre-cloacal median fin fold; pectoral fin is flattened and wide open mouth protruding anteriorly (Tsai et al., 2013).

Sex determination and differentiation

In fish, sex can be determined by mechanisms that are genetic, environmental, or a combination of both (Volff, 2005; Marshall Graves, 2008). Environmental factors that control sex determination in fish species include water temperature, density, and social interactions. Genetic control of sex determination is governed by the presence of sex chromosomes (visible sex chromosomes or heteromorphic chromosomes) that can be present either in males (XY) or in females (ZW).

N. furzeri has a genetic sex-determination system, with males as the heterogametic sex, indicative of an XY/XX system (Valenzano et al., 2009).

Medaka males and females differ by several secondary sex characters, some of which — such as the shape and size of the dorsal and anal fins — can be easily scored (Yamamoto, 1975). Additionally, strains with sex-linked pigmentation patterns have been established and are available (Tomita et al., 1975; Handler et al., 1993). Morphological development of the gonads in all vertebrate groups appears to have been conserved through evolution. Many genes that are important in gonadal sex differentiation in mammals, such as DMRT1, have been identified for the first time in medaka (Matsuda et al., 2002; Nanda et al., 2002), and show gonad-specific expression during the period of sexual differentiation. The identified gene, mrt1bY, homologous of the mammalian, is the male determining gene in the medakafish (Herpin and Schartl, 2011). Medaka has an XY–XX genetic sex determination system, with undifferentiated (homomorphic) sex chromosomes (Matsuda et al., 2002; Nanda et al., 2002). It is the first vertebrate where sex chromosomal inheritance and sex chromosomal crossovers have been described (Aida, 1921). The first morphological sex difference of gonads appears in the number of gonial-type germ cells one or two days before hatching (Iwamatsu, 2004). From this stage, the activity of germ cell division in XX embryos becomes higher than that of XY embryos, and then male germ cells arrest in mitosis (Kobayashi et al., 2004; Satoh and Egami,

1972). In males, somatic cells display an acinous structure, which is the precursor of the testicular seminiferous tubules and can be distinguished at 10 days after hatching (Kanamori et al., 1985). In females, ovarian follicles are the first female-specific structure and become evident around the diplotene oocytes about 20 days after hatching (Kanamori et al., 1985). After these structures have developed efferent ducts in the testes and ovarian cavities in ovaries become apparent. Furthermore, unlike in higher vertebrates, full sex reversals can be obtained in medaka. Treatment with steroid sex hormones during the larval period has generated YY males, XY females, XX males and even YY females 2. Such experiments uncovered an important phenomenon: sex can be artificially reverted as long as the gonad is morphologically indifferent (Wittbrodt et al., 2002). Finally, sex-specific pigmentation can be used to distinguish male from female embryos as early as 3 days postfertilization (dpf) (organogenesis stages) (Wada et al. 1998).

Zebrafish does not have a clear genetic basis of sex determination (von Hofsten and Olsson, 2005).

Goldfish possess an XX–XY sex determination system (Yamamoto and Kajishima, 1968). It is possible to control spawning, fertilization, and embryonic development by varying the water temperature and to manipulate both genetic and phenotypic sex by gynogenesis and temperature control (Yamaha et al., 1986, Yamaha et al., 1999, Goto-Kazeto et al., 2006).

Nervous system and sensory organs

Fish possess simple nervous systems compared to mammals, with far fewer neurons. However, they emerged as important model system for early patterning events and later events that build the three-dimensional structure of the brain. Genes that are important for fish brain patterning are usually conserved in mammals, and therefore relevant for understanding normal and abnormal human brain development and phisiology.

Fish brain is similar to other vertebrates, sharing many structural properties such as the main organization (fore-, mid- and hind-brain, including diencephalon, telencephalon and cerebellum), and the principal neurotransmitter systems. Despite smaller cerebral hemispheres and the structure and function of the optic tectum compared to mammals, it shows similarly defined areas such as the hypothalamus and olfactory bulb, encompassing structures of the lateral pallium (located in the telencephalon), which appear to be homologous to the mammalian hippocampus (Santana et al., 2012). However, it is also well known that a great interspecific diversity exists in brain morphology in teleosts (Meek

and Nieuwhenuyis, 1998). This diversity offers large opportunity to correlate ecology with brains and sensory systems.

Turquoise killifish and medaka both belong to the superorder Acantopterygii, and their brain structures (D'Angelo, 2012; Ishikawa et al., 1999) are strikingly different in several important features from those of cyprinids, including zebrafish and goldfish (Wullimann et al., 1996; Peter and Gill, 1975).

The morphology of adult brain turquoise killifish has been described (D'Angelo, 2012), showing a typical organization and subdivision of all teleosts central nervous system (CNS). Particularly, it has been observed well developped visual system structures, *e.g.* well prominent optic lobes, and glomerular nucleus. Also, glial cell population have been identified (D'Angelo et al., 2012), with the preminent localization along the ventricles and in the body of cerebellum. The brain is well suited for gene expression studies (D'Angelo et al., 2014) and proteins (D'Angelo et al., 2014). In course of aging, typical cellular phenotypes observed in the brain are: reduction of stem cells activity (Tozzini et al., 2012); gliosis and neuronal degeneration (Valenzano et al., 2006).

The anatomy of medaka brain has been studied (Ishikawa et al., 1999), and the morphogenesis has been studied based on a fate map and gene expression patterns (Hirose et al., 2004; Kage et al., 2004; Ishikawa et al., 2008).

Zebrafish has proven to be an excellent model organism to study neurogenesis in the embryo (Schmidt et al., 2013; 2014). Studies have shown that the adult zebrafish brain may also serve as a valuable model for the study of adult neurogenesis (Zupanc et al., 2005; Adolf et al., 2006). The brain of zebrafish is highly accessible during development, because of the larval transparency. This allows *in vivo* neuronal network analysis. Recently whole brain/single cell functional imaging techniques have been developed, enabling monitoring of neuronal activity in hundreds of neurons at once (Leung et al., 2013). A detailed description of the brain of zebrafih during development is available on http://www.zebrafishbrain.org/.

The description of CNS of goldfish represents one of the pioneering in a fish (Peter and Gill, 1975). The optic tectum is dome-shaped and continues to grow by the addition of sequential rings of new cells at the marginal portion called the peripheral growth zone (Raymond and Easter, 1983). Studies addressed to the localization of neurotransmitters and number of neuropeptides mainly related to neuroendocrine signaling have been carried out (Popesku et al., 2008) and represent a further useful tool for describing the morphology of brain structures and nuclei.

Neuroendocrine system

The pituitary gland in fish secretes a number of hormones, which affect growth, osmoregulation, lipid metabolism and reproductive development and behavior, as well as controlling other endocrine glands (Bone et al., 1995). One major difference in the anatomy of the mammalian versus teleostean hypothalamo-pituitary axis is the median eminence, which connects the pituitary with the hypothalamus. In mammals is a stalk-like neurohemal structure, transporting neuropeptides, whereas in all teleosts this structure does not exist and the pituitary is positioned directly underneath the hypothalamus.

Any morphological descriptions are available on the pituitary gland of turquoise killifish.

The pituitary gland of medaka has accurately been described by Aoki and Umeura (1970). It consists of three portions: the pars distalis, the pars intermedia and the neurohypophysis. 8 different cytotypes have been characterized on the basis tinctorial and histochemical properties. Transgenic models of medaka have been used to analyze the multisynaptic neuronal circuitry regulating the pituitary functions (Karigo et al., 2014).

The pituitary gland of zebrafish consists of two different parts, which differ in developmental origin and physiology. The neurohypophysis (posterior pituitary) derives from a ventral extension of the developing hypothalamus and represents the neural compartment of the gland. It consists of axonal nerve endings from hypothalamic magnocellular neurons, and pituicytes, which do not generate hormones but most likely have supportive and modulatory functions. Pituicytes can be readily identified by the expression of specific marker genes such as *fzd8b* and and *crap1b* (Löhr and Hammerschmidt, 2011). The adenohypophysis (anterior pituitary) constitutes the nonneural part and is embryologically derived from placodal ectoderm. It contains distinct endocrine cell lineages, which are characterized by the type of hormone they secrete. Nine different cell types can be distinguished on the basis of anatomical position and hormone expression profile: lactotropes, two distinct groups of corticotropes, thyrotropes, somatotropes, two groups of somatolactotropes, melanotropes, and gonadotropes (Löhr and Hammerschmidt, 2011). Zebrafish represents a powerful approach to elucidate developmental and physiological mecha- nisms of the endocrine system. Classical gain-of-function approaches like intraperitoneal and intracerebroventricular hormone injections, although possible, are technically much more challenging. For this purpose goldfish is more suitable.

Goldfish, indeed, has been using as an excellent model for neuroendocrine regulation of energy balance and reproduction (Bernier and Peter, 2001a; Popesku et al., 2008). The

pituitary gland consists of three lobes: rostral and proximal pars distalis, and the neuro-intermediate lobe. The rostral pars distalis is the smallest of the three lobes, occupies the dorsal area of the posterior part of the gland and is intimately related to the posterior aspect of the short and delicate pituitary stalk. It contains acidophilic, basophilic and chromophobic cells. The proximal pars distalis, the second pituitary lobe, was found beneath both the rostral pars distalis and the pituitary stalk. It extended to a varying degree into the anterior part of the gland and contained two types of basophilic and one type of acidophilic cell. In the largest pituitary lobe, the intrinsic cells did not exhibit conspicuous staining properties (Kaul and Vollrath, 1974a,b). Gonadotroph cells are clustered in the proximal pars distalis in association with somatotrophs (Ball, 1981), which allows for the precise determination of the preoptic telencephalic and hypothalamic origins of hypophysiotropic inputs tothe pituitary using tract-tracing methods (Anglade et al., 1993). The anterior pituitary gland is innervated by numerous neuronal cell types, and thus pituitary hormone release is directly regulated. Thanks to regionalized distribution of cells in the goldfish pituitary, it has been demonstrated a unique reciprocal paracrine relationship between gonadotrophs and somatotrophs, mediated by luteinizing hormone and growth hormone (Wong et al., 2007).

Sensory organs

The retina of teleosts consists of three nuclear layers and two plexiform layers. The outer nuclear layer contains the cell bodies of the photoreceptors (rods and cones). The inner nuclear layer contains the cell bodies of the horizontal, bipolar and amacrine cells, and the ganglion cell layer contains the ganglion cell bodies. The plexiform layers are found between the nuclear layers, where the synaptic connections between the retinal neurons take place. The outer plexiform layer (OPL) consists of the connections among photoreceptors, bipolar and horizontal cells, and the inner plexiform layer (IPL) consists of the connections among bipolar, amacrine and ganglion cells (Bilotta and Saszik, 2001). Few differences, ascribable to organization of cones and rods, have been described between medaka and zebrafish (Tohya et al., 2003). Since retina is a well anatomical conserved structure in Teleosts, zebrafish (Hoon et al., 2014), medaka (Conte et al., 2010), the turquoise killifish (Gatta et al., 2014) and goldfish (Braisted et al., 1994) have been using as models for addressing studies on development, physiology of visual system and perception (Rosa Salva et al., 2014), regeneration (Goldman, 2014), and diseases (Hoon et al., 2014).

Olphactory system

Fish olfactory system comprises only one main pathway, originating in the nasal cavity and giving rise to what is commonly referred to as the main olfactory system in mammals. Fish lack the other pathway, originating in the vomeronasal organ and giving rise to the accessory olfactory system (Dulka et al., 1993).

The nasal cavity of zebrafish is displaced by the nasal pit in zebrafish, a tubular structure, that opens to the exterior via anterior and posterior pores and has no communication with the oral cavity (Taniguchi and Taniguchi, 2014). In the nasal cavity, there are bilaterally, symmetric olfactory epithelia (OE) that are folded into rosette-shaped sensory organs. Each OE is connected via a short olfactory nerve to the olfactory bulbs. Three types of olfactory receptor neurons have been identified in the OE: ciliated, with round somata located deep in the epithelium and extended long, ciliated dendrites to the epithelial surface (Castro et al., 2006; Gayoso et al., 2011); microvillous, with various morphologies and differences in antibody labeling at intermediate depths in the OE; and cryptic, with ovoid shape, rounded apical pole, eccentric basal nucleus, and located near the surface of the sensory epithelium, labeled, among others, by S100 (Braubach et al., 2012; Parisi et al., 2014). Axons of olphactory neurons target the glomeruli in the olphactory bulbs. Glomeruli are organized in nine distinct regions reproducibly located on dorsal, ventral, lateral, and medial surfaces of the olfactory bulbs (Braubach et al., 2012).

The olfactory system of goldfish includes anatomical and functional subdivisions, that resemble those associated with the main and accessory olfactory systems in tetrapods, being particularly well suited for comparisons. The main reasons are:

- the olfactory pathways that regulate responses to sex pheromones in goldfish are different from those that serve a more general olfactory function;
- the functional differences seem to be subserved by separate and anatomically distinct olfactory tract projections to the brain;
- the lateral olfactory tracts and their central projections in goldfish appear to serve a function analogous to that of the main olfactory system, while the medial olfactory tracts and their central projections comprise a pathway similar to the vomeronasal-accessory olfactory system (Dulka, 1993).

Lateral line system

In fishes and amphibians, the lateral line is a superficial mechanosensory system, combining some structural and physiological characteristics of the mammalian vestibuloauditory and somatosensory systems. Lateral line may be complete, running from the head to the tail, or incomplete, starting at the head and ending before the tail.

In zebrafish, lateral line is a model for studying the coordination of cell migration and morphogenesis, in addition to its use for studying hair cell biology relating to human hearing and balance disorders (Whitfield, 2002; Nicolson, 2005). Lateral line system is made of peripheral receptors, mechanoreceptive neuromasts containing mechanosensory hair cells innervated by afferent and efferent neurons and surrounded by nonsensory support cells (Ghysen and Dambly-Chaudière, 2007; Bleckmann and Zelick, 2009). Hair cells locally acquire mechanical signals and transform it into chemical signals that are further converted into electrical impulses, transported to the brain by afferent neurons. Hair cells possess a mechano sensing organelle protruding from the cell's apical surface. In the neuromast, hair cells are contained within a gelatinous cupula that projects into the surrounding water, and are formed by an array of stereocilia arranged in rows of increasing length, and a kinocilium eccentrically located adjacent to the tallest stereocilia.

Cardiovascular system

In fishes a single heart circuit causes the blood to be directly routed through the entire organ in a posterior to anterior direction. In order, the primitive heart chambers are:

1) sinus venosus, a thin-walled distensible sac into which the venous blood is returned;

2) atrium, also thin-walled;

3) ventricle, the thick-walled major contractile portion of the heart;

4) conus arteriosus, a thick, but narrow tubular portion of the heart that is continuous with the ventral aorta. Hearts of fish, from the perspective of myocardial oxygen supply, have four main arrangements. The type I has entirely spongy myocardium and a cardiac circulation. The type II heart has an outer compact myocardium separated from the spongy myocardium by a layer of connective tissue. The coronary vessels in the compact myocardium do not penetrate the spongy myocardium. The type III and IV differ from the type II hearts because the connective tissue lacks and coronary vessels penetrate the spongy myocardium.

Any morphological data are available on the heart of turquoise killifish.

The heart morphology of medaka was studied by light and electron microscopy (Lemanski et al., 1975). The epicardial layer forms an outer covering over the organ and is composed of simple squamous epithelial cells. The ventricle is trabeculated, showing a "spongy" appearance; the atrium is less extensively trabeculated. The myocardial cells of the trabeculae have small diameters but extend for considerable distances. The myofibrils usually are located peripherally, while the nucleus, mitochondria, and other cellular

organelles are located centrally. The endocardium is composed of a continuous layer of cells that appear to be metabolically very active (Lemanski et al., 1975).

The cardiac morphology of developing (Hu et al., 2000) and adult (Hu et al., 2001) zebrafish has been described. Furthermore, several studies have been conducted to validate zebrafish as model for cardiovascular disease and vasculogenesis (Asnani and Peterson, 2014). The use of fluorescent reporters has been essential to identify two discrete phases of cardiomyocyte differentiation necessary for normal cardiac development in the zebrafish. These phases are analogous to the differentiation of cardiomiocytes in mammals, thus heart embryogenesis is conserved between zebrafish and mammals (Asnani and Peterson, 2014). Zebrafish has been established as model for discovering molecular mechanisms of human cardiovascular diseases, which includes prevalent forms of cardiomyopathy: dilated cardiomyopathy and hypertrophic cardiomyopathy. Many of the implicated genes in human cardiomyopathy such as titin (ttn) (Xu et al., 2002), tropomyosin (tpm4) (Zhao et al., 2008), troponin 2 (tnnt2) (Sehnert et al., 2002), myosin light chain (cmlc1, myl7) (Rottbauer et al., 2006) and myosin heavy chain (myh6) (Berdougo et al., 2003) have been mutated also in zebrafish, and causing cardiomyopathy, revealed by ultrastructural examination (Poon and Brand, 2013).

Goldfish possesses a type II heart that consists of a relatively thin vascularized compact heart and an extensive avascular spongy heart. In addition, goldfish displays a more saccular shaped heart, perhaps reflecting their specific ecological physiology (Grivas et al., 2014).

Urinary apparatus

The kidney in fish is located retroperitoneal, exterior to the dorsal wall of the body cavity. The kidney is a paired organ that has been described as having various anatomical and functional compartments (Morovvati et al., 2012). The kidney of fish receives majority of postbranchial blood and renal lesions may be expected to be good indicators of environmental stress. The head of kidney contains endocrine elements, the chromaffin cells and interregnal tissue, which are located around the blood vessels. The posterior kidney contains the nephrons with variable quantities of hemopoietic and lymphoid tissue in the interstitium.

In the kidney of medaka, the glomeruli are frequently found beneath the renal capsule, which consisted of fine connective tissue. Like mammals, each medaka glomerulus

exhibited a well developped glomerular capillary and an arborized mesangium in medaka adult (Ichimura et al., 2013).

There are several advantages in studying glomerular development in the medaka pronephric glomerulus compared to zebrafish and other teleosts (Ichimura et al., 2012). The glomerular primordium of the medaka pronephros exhibits a C-shaped epithelial layer. The C-shaped primordium contains a characteristic balloon-like capillary, which later divides into several smaller capillaries. A pair of pronephric glomeruli remains independent of each other due to the interposition of the mass of interglomerular mesangium (IGM) between them. The IGMCs possesses numerous cytoplasmic granules throughout pronephric development. The morphological process of podocyte differentiation in medaka is more similar to mammals (Ichimura et al., 2012). In particular, the glomerular primordium of the medaka pronephros exhibits a C-shaped epithelial layer of primitive podocytes, which is similar to that of mammalian S-shaped body.

The adult zebrafish kidney, or mesonephros, is a single, relatively flat organ attached to the dorsal body wall that consists of characteristic bilaterally symmetric regions referred to as the head (or anterior), trunk (or medial), and tail (or posterior) (Gerlach et al., 2011).

Table 1.

Main field of use		Turquoise killifish	Medaka	Zebrafish	Goldfish
Anatomy and physiology		Basic research (age research)	Basic research (developmenta l biology)	Basic research (developmental biology)	Basic research (neuro-endocrine studies)
Applied pharmacology		Drug validation for aging (Valenzano and Cellerino, 2006)	Anticancer drug (Matsuzaki et al., 2013)	General use (Haesemeyer and Schier, 2015)	
Pathology	*Cancer and cancerogenesis*	Spontaneous tumors in brain, liver and genital apparatus (Di Cicco et al., 2011)	Spontaneous lymphoma, ovarian tumour; induced melanoma, liver tumors, and xenograft (Hasegawa et al., 2009)	Spontaneous and induced tumors, and through transgenesis* and xenograft (White et al., 2013).	
	Toxicology		Ecotoxicity/toxi city tests (Padilla et al., 2009)	Ecotoxicity/ drug development (Gaytán and Vulpe, 2014)	Ecotoxicity (Velma and Tchounwou, 2011)
	Neuro-degeneration and neuropathology	Neuro-degeneration (Valenzano et al., 2006)	Retinite pigmentosa (Conte et al., 2015)	Genetic-based pathologies (Newman et al., 2014)	
	Endocrinology and endocrine pathologies			Diabetes. Food intake regulation (Matsuda et al., 2012)	Hypothalamic-pituitary axis. Food intake regulation (Popesku et al., 2008).
	Genetic pathologies		General use (Schartl, 2014)	General use (Schartl, 2014)	
	Others			Cardiovascular diseases (Asnani and Peterson, 2014).	

* melanoma, pancreatic tumors, T cell lymphoma or leukaemia, B cell leukaemia, rhabdomyosarcoma, neuroblastoma, lipoma, Ewing's sarcoma, myeloproliferative neoplasms, corticotroph adenoma and neoplasm, testicular germ cell tumour.

A summary of the utilization of different fish species in current biomedical research.

Table 2.

	Turquoise killifish	Medaka	Zebrafish	Goldfish
Embryonic development	Hatching 12 days after fertilization, as indipendent feeding larva	Hatching9 days after fertilization, as indipendent feeding larva	Hatching 3 days after fertilization, indipendent feeding after 5 days post fertilization	Hatching 3 days after fertilization, indipendent feeding after 5 days post fertilization
Digestive apparatus	Oral and pharyngeal teeth. Stomach	Oral and pharyngeal teeth. Stomachless	Teeth attached to the fifth branchial arch. Stomachless	Pharyngeal teeth. Stomachless
Reproductive system	Sexual dimorphism	Sexual dimorphism	Sexual dimorphism	Sexual dimorphism
Skin	Highly colourful skin in male.	Pigmented skin. 4 types of cromatophores	Five uniformly, pigmented, horizontal stripes on the side of the body	Head without scales
Lateral line	Complete	Subdivided in anterior and posterior lateral line, according to the neuromasts position	Subdivided in anterior and posterior lateral line, according to the neuromasts position	Complete

General anatomical features of the most used fish in biomedical research.

References

Adolf, B., Chapouton, P., Lam, C.S., Topp, S., Tannhäuser, B., Strähle, U., Götz, M., Bally-Cuif, L., 2006. Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. Dev Biol. 295(1),278-93.

Aida, T., 1921. On the Inheritance of Color in a Fresh-Water Fish, APLOCHEILUS LATIPES Temmick and Schlegel, with Special Reference to Sex-Linked Inheritance. Genetics. 6(6),554-73.

Anglade, I., Zandbergen, T., Kah, O. 1993. Origin of the pituitary innervation in the goldfish.Cell Tissue Res. 273(2),345-55.

Aoki, K., Umeura, H., 1970. Cell types in the pituitary of the medaka, Oryzias latipes. Endocrinol Jpn. 17(1):45-55.

Aparicio, S., Chapman, J., Stupka, E., Putnam, N., Chia, J.M., Dehal, P., Christoffels, A., Rash, S., Hoon, S., Smit, A., Gelpke, M.D., Roach, J., Oh, T., Ho, I.Y., Wong, M., Detter, C., Verhoef, F., Predki, P., Tay, A., Lucas, S., Richardson, P., Smith, S.F., Clark, M.S., Edwards, Y.J., Doggett, N., Zharkikh, A., Tavtigian, S.V., Pruss, D., Barnstead, M., Evans, C., Baden, H., Powell, J., Glusman, G., Rowen, L., Hood, L., Tan, Y.H., Elgar, G., Hawkins, T., Venkatesh, B., Rokhsar, D., Brenner, S. 2002. Whole-genome shotgun assembly and analysis of the genome of Fugu rubripes. Science 297(5585):1301-10.

Argenton, F., Zecchin, E., Bortolussi, M., 1999. Early appearance of pancreatic hormone-expressing cells in the zebrafish embryo. Mech Dev. 87(1-2),217-21.

Asnani, A., Peterson, R.T., 2014. The zebrafish as a tool to identify novel therapies for human cardiovascular disease. Dis Model Mech. 7(7),763-7.

Ball, J.N., 1981. Hypothalamic control of the pars distalis in fishes, amphibians, and reptiles. Gen Comp Endocrinol. 44(2), 135-70.

Barrington, E.J.W., 1957. The alimentary canal and digestion. In The physiology of fishes Volume 1. Edited by Brown ME. Academic Press, New York, 109-161.

Berdougo, E., Coleman, H., Lee, D.H., Stainier, D.Y., Yelon, D. 2003. Mutation of weak atrium/atrial myosin heavy chain disrupts atrial function and influences ventricular morphogenesis in zebrafish. Development 130, 6121–6129.

Bernier, N.J., Peter, R.E., 2001. Appetite-suppressing effects of urotensin I and corticotropin-releasing hormone in goldfish (Carassius auratus). Neuroendocrinology 73(4), 248-60.

Bernier, N.J., Peter, R.E., 2001a. T he hypothalamic-pituitary-interrenal axis and the control of food intake in teleost fish. Comp Biochem Physiol B Biochem Mol Biol. 129(2-3), 639-44.

Bilotta, J., Saszik, S., 2001. The zebrafish as a model visual system. Int J Dev Neurosci. 19(7), 621-9.

Blažek, R., Polačik, M., Reichard, M., 2013. Rapid growth, early maturation and short generation time in African annual fishes. Evodevo. 4(1), 24.

Bleckmann, H., Zelick,R., 2009. Lateral line system of fish. Integr. Zool. 4,13–25.

Bone, Q., 1978. Locomotor muscle. In Fish Physiology (ed. W. S. Hoar and D. J. Randall), Vol. 7, pp. 361-424. New York: Academic Press.

Bone, Q., Marshall, N.B., Blaxter, J.H.S., 1995. Biology of Fishes. Chapman and Hall, New York. 332pp.

Bowden, T.J., Cook, P., Rombout,J.H.W.M., 2005. Development and function of the thymus in teleosts. Fish & Shellfish Immunology, vol. 19, no. 5, pp. 413–427.

Braisted, J.E., Essman, T.F., Raymond, P.A., 1994. Selective regeneration of photoreceptors in goldfish retina. Development 120(9), 2409-19.

Braubach, O.R., Fine, A., Croll, R.P., 2012. Distribution and functional organization of glomeruli in the olfactory bulbs of zebrafish (Danio rerio). J Comp Neurol. 520(11), 2317-39.

Castro, A., Becerra, M., Manso, M.J., Anadón, R. 2006. Calretinin immunoreactivity in the brain of the zebrafish, Danio rerio: distribution and comparison with some neuropeptides and neurotransmitter-synthesizing enzymes. I. Olfactory organ and forebrain. J Comp Neurol. 494(3), 435-59.

Cellerino, A., Valenzano, D.R., Reichard, M., 2015. From the bush to the bench: the annual Nothobranchius fishes as a new model system in biology. Biol Rev Camb Philos Soc. doi: 10.1111/brv.12183.

Chen, S., Li, C., Yuan, G., Xie, F. 2007. Anatomical and histological observation on the pancreas in adult zebrafish. Pancreas 34(1),120-5.

Cheng, L., Chang, Y.M., Lu, C.Y., Cao, D.C., Sun, X.W. 2012. DNA barcoding and species and subspecies classification within genus Carassius sp. Zoological Research 33: 463-472.

Christoffels, A., Koh, E.G., Chia, J.M., Brenner, S., Aparicio, S., Venkatesh, B. 2004. Fugu genome analysis provides evidence for a whole-genome duplication early during the evolution of ray-finned fishes. Mol Biol Evol. 21(6), 1146-51.

Conte, I., Hadfield, K.D., Barbato, S., Carrella, S., Pizzo, M., Bhat, R.S., Carissimo, A., Karali, M., Porter, L.F., Urquhart, J., Hateley, S., O'Sullivan, J., Manson, F.D., Neuhauss, S.C., Banfi, S., Black, G.C., 2015. MiR-204 is responsible for inherited retinal dystrophy associated with ocular coloboma. Proc Natl Acad Sci U S A. 112(25):E3236-45.

Conte, I., Marco-Ferreres, R., Beccari, L., Cisneros,E., Ruiz, J.M., Tabanera, N., Bovolenta, P., 2010. Proper differentiation of photoreceptors and amacrine cells depends on a regulatory loop between NeuroD and Six6. Development 137(14), 2307-17.

D'Angelo, L. 2012 Brain atlas of an emerging teleostean model: Nothobranchius furzeri. Anat Rec (Hoboken). 296(4),681-91.

D'Angelo, L., Castaldo, L., Cellerino, A., de Girolamo, P., Lucini, C., 2014. Nerve growth factor in the adult brain of a teleostean model for aging research: Nothobranchius furzeri. Ann Anat. 196(4),183-91.

D'Angelo, L., De Girolamo, P., Cellerino, A., Tozzini, E.T., Varricchio, E., Castaldo, L., Lucini, C., 2012. Immunolocalization of S100-like protein in the brain of an emerging model organism: Nothobranchius furzeri. Microsc Res Tech. 75(4),441-7.

D'Angelo, L., De Girolamo, P., Lucini, C., Terzibasi, E.T., Baumgart, M., Castaldo, L., Cellerino, A, 2014. Brain-derived neurotrophic factor: mRNA expression and protein distribution in the brain of the teleost Nothobranchius furzeri. J Comp Neurol. 522(5),1004-30.

Davidson, A.J., Zon, L.I., 2004. The 'definitive' (and 'primitive') guide to zebrafish hematopoiesis.Oncogene vol. 23, no. 43, pp. 7233–7246.

De Pedro, N., Alonso-Gómez, A.L., Gancedo, B., Delgado, M.J., Alonso-Bedate, M., 1993. Role of corticotropin-releasing factor (CRF) as a food intake regulator in goldfish.Physiol Behav. 53(3), 517-20.

Debiais-Thibaud, M., Borday-Birraux, V., Germon, I., Bourrat, F., Metcalfe, C.J., Casane, D., Laurenti, P., 2007. Development of oral and pharyngeal teeth in the medaka (Oryzias latipes): comparison of morphology and expression of eve1 gene. J Exp Zool B Mol Dev Evol. 308(6), 693-708.

Di Cicco, E., Tozzini, E.T., Rossi, G., Cellerino, A., 2011. The short-lived annual fish Nothobranchius furzeri shows a typical teleost aging process reinforced by high incidence of age-dependent neoplasias. Exp Gerontol. 46(4):249-56.

Dulka, J.G., 1993. Sex pheromone systems in goldfish: comparisons to vomeronasal systems in tetrapods. Brain Behav Evol. 42(4-5):265-80.

Evans, D.H., Piermarini, P.M., Choe, K.P., 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev. 85(1):97-177.

Furutani-Seiki, M., Wittbrodt, J., 2004. Medaka and zebrafish, an evolutionary twin study. Mech Dev. 121(7-8), 629-37.

Gatta, C., Castaldo, L., Cellerino, A., de Girolamo, P., Lucini, C., D'Angelo, L., 2014. Brain derived neurotrophic factor in the retina of the teleost N. furzeri. Ann Anat. 196(4),192-6.

Gayoso, J.Á., Castro, A., Anadón, R., Manso, M.J., 2011. Differential bulbar and extrabulbar projections of diverse olfactory receptor neuron populations in the adult zebrafish (Danio rerio). J Comp Neurol. 519(2), 247-76.

Gaytán, B.D., Vulpe, C.D., 2014. Functional toxicology: tools to advance the future of toxicity testing. Front Genet. 5:110.

Genade, T., Benedetti, M., Terzibasi, E., Roncaglia, P., Valenzano, D.R., Cattaneo, A., Cellerino, A., 2005. Annual fishes of the genus Nothobranchius as a model system for aging research. Aging Cell. 4(5), 223-33.

Gerlach, G.F., Schrader, L.N., Wingert, R.A. 2011. Dissection of the adult zebrafish kidney. J Vis Exp. (54).

Ghysen A., Dambly-Chaudière, C., 2007. The lateral line microcosmos. Genes Dev. 21,2118–2130.

Goldman, D., 2014. Müller glial cell reprogramming and retina regeneration. Nat Rev Neurosci. 15(7):431-42.

Goto-Kazeto, R., Abe, Y., Masai, K., Yamaha, E., Adachi, S., Yamauchi, K. 2006. Temperature-dependent sex differentiation in goldfish: Establishing the temperature-sensitive period and effect of constant and fluctuating water temperatures. Aquaculture, 254:617–624

Graham, C.F., Morgna, R.W., 1966. Changes in the cell cycle during early amphibian development.Developmental Biology 14, 439–460.

Grivas, J., Haag, M., Johnson, A., Manalo, T., Roell, J., Das, T.L., Brown, E., Burns, A.R., Lafontant, P.J., 2014. Cardiac repair and regenerative potential in the goldfish (Carassius auratus) heart. Comp Biochem Physiol C Toxicol Pharmacol. 163:14-23.

Haesemeyer, M., Schier, A.F., 2015. The study of psychiatric disease genes and drugs in zebrafish. Curr Opin Neurobiol. 30:122-30.

Haffter, P., Odenthal, J., Mullins, M.C., Lin, S., Farrell, M.J., Vogelsang, E., Haas, F., Brand, M., van Eeden, F.J., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C.P., Jiang, Y.J., Kane, D.A., Kelsh, R.N., Hopkins, N., Nüsslein-Volhard, C., 1996. Mutations affecting pigmentation and shape of the adult zebrafish. Dev Genes Evol. 206(4), 260-76.

Handler, A.M., Gomez, S.P., O'Brochta, D.A., 1993. A functional analysis of the P-element gene-transfer vector in insects. Arch. Insect Biochem. Physiol. 22, 373–384.

Harder, W., 1975, Anatomy of fishes. Part I. Text. Part 2. Figures and plates. Stuttgart. E. Schweizerbart'sche Verlagsbuchhandlung, Pt.1:612 p., Pt.2:132 p. 13 pl.

Hartmann, N., Englert, C., 2012. A microinjection protocol for the generation of transgenic killifish (Species: Nothobranchius furzeri). Dev Dyn. 241(6), 1133-41.

Hasegawa, S., Maruyama, K., Takenaka, H., Furukawa, T., Saga, T., 2009. A medaka model of cancer allowing direct observation of transplanted tumor cells in vivo at a cellular-level resolution. Proc Natl Acad Sci U S A. 106(33):13832-7.

Herpin, A., Schartl, M., 2011. Molecular mechanisms of sex determination and evolution of the Y-chromosome: insights from the medakafish (Oryzias latipes). Mol Cell Endocrinol. 306(1-2),51-8.

Hesselson, D., Anderson, R.M., Beinat, M., Stainier, D.Y., 2009. Distinct populations of quiescent and proliferative pancreatic beta-cells identified by HOTcre mediated labeling. Proc Natl Acad Sci U S A. 106(35), 14896-901.

Hirose, Y., Varga, Z.M., Kondoh, H., Furutani-Seiki, M., 2004. Single cell lineage and regionalization of cell populations during Medaka neurulation. Development. 131(11):2553-63.

Hoon, M., Okawa, H., Della Santina, L., Wong, R.O., 2014. Functional architecture of the retina: development and disease. Prog Retin Eye Res. 42, 44-84.

Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J.C., Koch, R., Rauch, G.J., White, S., Chow, W., Kilian, B., Quintais, L.T., Guerra-Assunção, J.A., Zhou, Y., Gu, Y., Yen, J., Vogel, J.H., Eyre, T., Redmond, S., Banerjee, R., Chi, J., Fu, B., Langley, E., Maguire, S.F., Laird,G.K., Lloyd, D., Kenyon, E., Donaldson, S., Sehra, H., Almeida-King, J., Loveland, J., Trevanion, S., Jones, M., Quail, M., Willey, D., Hunt, A., Burton, J., Sims, S., McLay, K., Plumb, B., Davis, J., Clee, C., Oliver, K., Clark, R., Riddle, C., Elliot, D., Threadgold, G., Harden, G., Ware, D., Begum, S., Mortimore, B., Kerry, G., Heath, P., Phillimore, B., Tracey, A., Corby, N., Dunn, M., Johnson, C., Wood, J., Clark, S., Pelan, S., Griffiths, G., Smith, M., Glithero, R., Howden, P., Barker, N., Lloyd, C., Stevens, C., Harley, J., Holt, K., Panagiotidis, G., Lovell, J., Beasley, H., Henderson, C., Gordon, D., Auger, K., Wright, D., Collins, J., Raisen, C., Dyer, L., Leung, K., Robertson, L., Ambridge, K., Leongamornlert, D., McGuire, S., Gilderthorp, R., Griffiths, C., Manthravadi, D., Nichol, S., Barker, G., Whitehead, S., Kay, M., Brown, J., Murnane, C., Gray, E., Humphries, M., Sycamore, N., Barker, D., Saunders, D., Wallis, J., Babbage, A., Hammond, S., Mashreghi-Mohammadi, M., Barr, L., Martin, S., Wray, P., Ellington, A., Matthews, N., Ellwood, M., Woodmansey, R., Clark, G., Cooper, J., Tromans, A., Grafham, D., Skuce, C., Pandian, R., Andrews, R., Harrison, E., Kimberley, A., Garnett, J., Fosker, N., Hall, R., Garner, P., Kelly, D., Bird, C., Palmer, S., Gehring, I., Berger, A., Dooley, C.M., Ersan-Ürün, Z., Eser, C., Geiger, H., Geisler, M., Karotki, L., Kirn, A., Konantz, J., Konantz, M., Oberländer, M., Rudolph-Geiger, S., Teucke, M., Lanz, C., Raddatz, G., Osoegawa, K., Zhu, B., Rapp, A., Widaa, S., Langford, C., Yang, F., Schuster, SC., Carter, N.P., Harrow, J., Ning, Z., Herrero, J., Searle, S.M., Enright, A, Geisler, R., Plasterk, R.H., Lee, C., Westerfield, M., de Jong, P.J., Zon, L.I., Postlethwait, J.H., Nüsslein-Volhard, C., Hubbard, T.J., Roest Crollius, H., Rogers, J., Stemple, D.L., 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 496(7446), 498-503

Hoyle, G.,1983. Muscles and their neural control. In Muscles and their Neural Control, 263-311. New York: John Wiley and Sons.

Hsu, C.Y., Chiu, Y.C., 2009. Ambient temperature influences aging in an annual fish (Nothobranchius rachovii). Aging Cell 8,726-737.

Hu, N,, Yost, H.J., Clark, E.B., 2001. Cardiac morphology and blood pressure in the adult zebrafish. Anat Rec. 264(1):1-12.

Hsu, H.H., Lin, L.Y., Tseng, Y.C., Horng, J.L., Hwang, P.P., 2014. A new model for fish ion regulation: identification of ionocytes in freshwater- and seawater-acclimated medaka (Oryzias latipes). Cell Tissue Res. 357(1):225-43.

Hu, N., Sedmera, D., Yost, H.J., Clark, E.B., 2000. Structure and function of the developing zebrafish heart. Anat Rec. 260(2):148-57.

Ichimura, K., Bubenshchikova, E., Powell, R., Fukuyo, Y., Nakamura, T., Tran, U., Oda, S., Tanaka, M., Wessely, O., Kurihara, H., Sakai, T., Obara, T., 2012. A comparative analysis of glomerulus development in the pronephros of medaka and zebrafish. PLoS One. 7(9), e45286.

Ichimura, K., Kawashima, Y., Nakamura, T., Powell, R., Hidoh, Y., Terai, S., Sakaida, I., Kodera, Y., Tsuji, T., Ma, J.X., Sakai, T., Matsumoto, H., Obara, T., 2013. Medaka fish, Oryzias latipes, as a model for human obesity-related glomerulopathy. Biochem Biophys Res Commun. 431(4):712-7.

Imai, S., Sasaki, T., Shimizu, A., Asakawa, S., Hori, H., Shimizu, N., 2007. The genome size evolution of medaka (Oryzias latipes) and fugu (Takifugu rubripes). Genes Genet Syst. 82(2):135-44.

Inohaya, K., Takano, Y., Kudo, A., 2007. The teleost intervertebral region acts as a growth center of the centrum: in vivo visualization of osteoblasts and their progenitors in transgenic fish. Dev Dyn. 236(11), 3031-46.

Ishikawa, Y., Yasuda, T., Kage, T., Takashima, S., Yoshimoto, M., Yamamoto, N., Maruyama, K., Takeda, H., Ito, H., 2008. Early development of the cerebellum in teleost fishes: a study based on gene expression patterns and histology in the medaka embryo. Zoolog Sci. 25(4):407-18.

Ishikawa, Y., Yoshimoto, M., Yamamoto, N., Ito, H., 1999. Different brain morphologies from different genotypes in a single teleost species, the medaka (Oryzias latipes). Brain Behav Evol. 53(1):2-9.

Iwamatsu, T., 2004. Stages of normal development in the medaka Oryzias latipes. Mech Dev. 121(7-8), 605-18.

Jagadeeswaran, P., Sheehan, J.P., Craig, F.E., Troyer, D., 1999. Identification and characterization of zebrafish thrombocytes. Br. J. Haematol. 107, 731–738.

Jaillon, O., Aury, J.M., Brunet, F., Petit, J.L., Stange-Thomann, N., Mauceli, E., Bouneau, L., Fischer, C., Ozouf-Costaz, C., Bernot, A., Nicaud, S., Jaffe, D., Fisher, S., Lutfalla, G., Dossat, C., Segurens, B., Dasilva, C., Salanoubat, M., Levy, M., Boudet, N., Castellano, S., Anthouard, V., Jubin, C., Castelli, V., Katinka, M., Vacherie, B., Biémont, C., Skalli, Z., Cattolico, L., Poulain, J., De Berardinis, V., Cruaud, C., Duprat, S., Brottier, P., Coutanceau, J.P., Gouzy, J., Parra, G., Lardier, G., Chapple, C., McKernan, K.J., McEwan, P., Bosak, S., Kellis, M., Volff, J.N., Guigó, R., Zody, M.C., Mesirov, J., Lindblad-Toh, K., Birren, B., Nusbaum, C., Kahn, D., Robinson-Rechavi, M., Laudet, V., Schachter, V., Quétier, F., Saurin, W., Scarpelli, C., Wincker, P., Lander, E.S., Weissenbach, J., Roest Crollius, H., 2004. Genome duplication in the teleost fish Tetraodon nigroviridis reveals the early vertebrate proto-karyotype. Nature 431(7011):946-57.

Johnston, I.A., Moon, T.W., 1981. Fine structure and metabolism of multiply innervated fast muscle fibres in teleost fish. Cell Tissue Res. 219(1):93-109.

Jonz, M.G., Buck, L.T., Perry, S.F., Schwerte, T., Zaccone, G., 2015. Sensing and surviving hypoxia in vertebrates. Ann N Y Acad Sci. doi: 10.1111/nyas.12780.

Jorge, H.U., Manuel, V., Susana, M., Ania, P.Q., Paulino, M., 2012. Development and validation of a molecular tool for assessing triploidy in turbot (Scophthalmus maximus). Aquaculture 380-383: 179-184.

Kage, T., Takeda, H., Yasuda, T., Maruyama, K., Yamamoto, N., Yoshimoto, M., Araki, K., Inohaya, K., Okamoto, H., Yasumasu, S., Watanabe, K., Ito, H., Ishikawa, Y. 2004. Morphogenesis and regionalization of the medaka embryonic brain. J Comp Neurol. 476(3):219-39.

Kalous, L., Bohlen, J., Rylková, K., Petrtýl, M. 2012. Hidden diversity within the Prussian carp and designation of a neotype for Carassius gibelio (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters 23: 11-18.

Kanamori, A., Nagahama, Y., Egami, N., 1985. Development of the tissue architecture in the gonads of the medaka, Oryzias latipes. Zool. Sci. 2, 695–706.

Karigo, T., Aikawa, M., Kondo, C., Abe, H., Kanda, S., Oka, Y., 2014. Whole brain-pituitary in vitro preparation of the transgenic medaka (Oryzias latipes) as a tool for analyzing the differential regulatory mechanisms of LH and FSH release. Endocrinology 155(2):536-47.

Kasahara, M., Naruse, K., Sasaki, S., Nakatani, Y., Qu, W., Ahsan, B., Yamada, T., Nagayasu, Y., Doi, K., Kasai, Y., Jindo, T., Kobayashi, D., Shimada, A., Toyoda, A., Kuroki, Y., Fujiyama, A., Sasaki, T., Shimizu, A., Asakawa, S., Shimizu, N., Hashimoto, S., Yang, J., Lee, Y., Matsushima, K., Sugano, S., Sakaizumi, M., Narita, T., Ohishi, K., Haga, S., Ohta, F., Nomoto, H., Nogata, K., Morishita, T., Endo, T., Shin-I, T., Takeda, H., Morishita, S., Kohara, Y., 2007. The medaka draft genome and insights into vertebrate genome evolution. Nature 447(7145):714-9.

Kaul S, Vollrath L., 1974(a). The goldfish pituitary. I. Cytology. Cell Tissue Res. 154(2):211-30.

Kaul S, Vollrath L., 1974(b). The goldfish pituitary. II. Innervation. Cell Tissue Res. 154(2):231-49

Kobayashi, D., Jindo, T., Naruse, K., Takeda, H., 2006. Development of the endoderm and gut in medaka, Oryzias latipes. Dev Growth Differ. 48(5):283-95.

Kobayashi, T., Matsuda, M., Kajiura-Kobayashi, H., Suzuki, A., Saito, N., 2004. Two DM domain genes, DMY and DMRT1, involved in testicular differentiation and development in the medaka, Oryzias latipes. Dev. Dyn. 231, 518–26.

Lam, S.H., Chua, H.L., Gong, Z., Wen, Z., Lam, T.J., Sin, Y.M., 2002. Morphologic transformation of the thymus in developing zebrafish. Dev Dyn. 225(1), 87-94.

Laurent, P., 1984. Morphology and physiology of organs of aquatic respiration in vertebrates: the gill. J Physiol. 79(2), 98-112.

Lemanski, L.F., Fitts, E.P., Marx, BS., 1975. Fine structure of the heart in the Japanese Medaka, Oryzias latipes. J Ultrastruct Res. 53(1):37-65.

Leung, L.C., Wang, G.X., Mourrain, P., 2013. Imaging zebrafish neural circuitry from whole brain to synapse. Front Neural Circuits 7, 76.

Levels, P.J., Gubbels, R.E., Denuce, J.M., 1986. Oxygen consumption during embryonic development of the annual fish Nothobranchius korthausae with special reference to diapause. Comparative Biochemistry and Physiology - Part A Molecular & Integrative Physiology 84, 767–770.

Lieschke, G.J., Currie, P.D., 2007. Animal models of human disease: zebrafish swim into view. Nat Rev Genet. 8(5), 353-67.

Lieschke, G.J., Trede, N.S., 2009. Fish immunology. Curr Biol, 19 (16) R678–R682

Löhr, H., Hammerschmidt, M., 2011. Zebrafish in endocrine systems: recent advances and implications for human disease. Annu Rev Physiol. 73:183-211.

Lossi, L., D'Angelo, L., de Girolamo, P., Merighi, A., 2015. Anatomical features for an adequate choice of the experimental animal model in biomedicine: II.Small laboratory rodents, rabbit, and pig. Ann. Anat. 2015.

Lucas-Sánchez, A., Almaida-Pagán, P.F., Madrid Pérez, J.A, de Costa Ruiz, J., Mendiola López, P., 2011. Age-related markers in Nothobranchius korthausae: fatty acid profile and locomotor activity rhythms. Exp Gerontol, 46,970-978.

Lucas-Sánchez, A., Almaida-Pagán, P.F., Mendiola, P., de Costa, J., 2014. Nothobranchius as a model for aging studies. Aging Dis. 5(4),281-91.

Marshall Graves, J.A., 2008. Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. Annu Rev Genet. 42:565-86.

Matsuda, K., Sakashita, A., Yokobori, E., Azuma, M., 2012. Neuroendocrine control of feeding behavior and psychomotor activity by neuropeptide Y in fish. Neuropeptides. 46(6):275-83.

Matsuda, M., Nagahama, Y., Shinomiya, A., Sato, T., Matsuda, C., Kobayashi, T., Morrey, C.E., Shibata, N., Asakawa, S., Shimizu, N., Hori, H., Hamaguchi, S., Sakaizumi, M., 2002. DMY is a Y-specific DM-domain gene required for male development in the medaka fish. Nature 417(6888), 559-63.

Matsuzaki, Y., Hosokai, H., Mizuguchi, Y., Fukamachi, S., Shimizu, A., Saya, H., 2013. Establishment of HRASG12V Transgenic Medaka as a Stable Tumor Model for In Vivo Screening of Anticancer Drugs. PLoS One. 8(1):e54424.

Meek, J., Nieuwenhuys, R., 1998. “Holosteans and teleosts,” in The Central Nervous System of Vertebrates, Vol. 2, eds Nieuwenhuys R., ten Donkelaar H. J., Nicholson C., editors. (Berlin: Springer;), 759–937.

Meyer, A., Schartl, M., 1999. Gene and genome duplications in vertebrates: the one-to-four (-to-eight in fish) rule and the evolution of novel gene functions. Curr Opin Cell Biol.11(6), 699-704.

Morovvati, H., Mahabady, M.K., Shahbazi, S., 2012. Histomorphological and anatomical study of kidney in berzem (Barbus pectoralis). International Journal of Fisheries and Aquaculture 4(11), 221-227.

Nanda, I., Kondo, M., Hornung, U., Asakawa, S., Winkler, C., Shimizu, A., Shan, Z., Haaf, T., Shimizu, N., Shima, A., Schmid, M., Schartl, M., 2002. A duplicated copy of DMRT1 in the sex-determining region of the Y chromosome of the medaka, Oryzias latipes. Proc Natl Acad Sci U S A.99 (18), 11778-83.

Newman, M., Ebrahimie, E., Lardelli, M., 2014. Using the zebrafish model for Alzheimer's disease research. Front Genet. 5:189.

Nicolson, T., 2005.The genetics of hearing and balance in zebrafish. Annu Rev Genet. 39, 9-22.

O'Reilly-Pol, T., Johnson, S.L., 2008. Melanocyte regeneration reveals mechanisms of adult stem cell regulation. Semin Cell Dev Biol. 20(1), 117-24.

Padilla, S., Cowden, J., Hinton, D.E., Yuen, B., Law, S., Kullman, S.W., Johnson, R., Hardman, R.C., Flynn, K., Au, D.W. 2009. Use of medaka in toxicity testing. Curr Protoc Toxicol. Chapter 1:Unit1.10.

Parisi, V., Guerrera, M.C., Abbate, F., Garcia-Suarez, O., Viña, E., Vega, J.A., Germanà, A., 2014. Immunohistochemical characterization of the crypt neurons in the olfactory epithelium of adult zebrafish. Ann Anat. 196(4), 178-82.

Peter, R.E., Gill, V.E., 1975. A stereotaxic atlas and technique for forebrain nuclei of the goldfish, Carassius auratus. J. Comp. Neurol. 159, 69–101.

Petzold, A., Reichwald, K., Groth, M., Taudien, S., Hartmann, N., Priebe, S., Shagin, D., Englert, C., Platzer, M., 2013. The transcript catalogue of the short-lived fish Nothobranchius furzeri provides insights into age-dependent changes of mRNA levels. BMC Genomics. 14:185.

Polačik, M., Donner, M.T., Reichard, M., 2011. Age structure of annual Nothobranchius fishes in Mozambique: is there a hatching synchrony? J Fish Biol. 78(3), 796-809.

Poon, K.L., Brand T., 2013. The zebrafish model system in cardiovascular research: A tiny fish with mighty prospects. Glob Cardiol Sci Pract. (1), 9-28.

Popesku, J.T., Martyniuk, C.J., Mennigen, J., Xiong, H., Zhang, D., Xia, X., Cossins, A.R., Trudeau, V.L., 2008. The goldfish (Carassius auratus) as a model for neuroendocrine signaling.Mol Cell Endocrinol.293(1-2), 43-56.

Rauta, PR., Nayak, B., Das, S., 2012. Immune system and immune responses in fish and their role in comparative immunity study: a model for higher organisms. Immunology Letters, 148 (1),23–33.

Raymond, P.A., Easter, S.S. Jr., 1983. Postembryonic growth of the optic tectum in goldfish. I. Location of germinal cells and numbers of neurons produced. J Neurosci 3: 1077–1091.

Reichwald, K., Lauber, C., Nanda, I., Kirschner, J., Hartmann, N., Schories, S., Gausmann, U., Taudien, S., Schilhabel, M.B., Szafranski, K., Glöckner, G., Schmid, M., Cellerino, A., Schartl, M., Englert, C., Platzer, M., 2009. High tandem repeat content in the genome of the short-lived annual fish Nothobranchius furzeri: a new vertebrate model for aging research. Genome Biol. 10(2):R16.

Rembold, M., Wittbrodt, J., 2004. In vivo time-lapse imaging in medaka--n-heptanol blocks contractile rhythmical movements. Mech Dev. 121(7-8),965-70.

Renshaw, S.A., Trede, N.S., 2012. A model 450 million years in the making: zebrafish and vertebrate immunity. Dis Model Mech 5 (1), 38–47.

Rossi, G., Messina, G., 2014. Comparative myogenesis in teleosts and mammals. Cell Mol Life Sci. 71(16), 3081-99.

Rottbauer, W., Wessels, G., Dahme, T., Just, S., Trano, N., Hassel, D., Burns, C.G., Katus, H.A., 2006. Cardiac myosin light chain-2: a novel essential component of thick-myofilament assembly and contractility of the heart. Circ Res. 99:323–331.

Russell, W.M.S., Burch, R.L., 1959. The Principles of Humane Experimental Technique.Methuen, London.

Russo, F., de Girolamo, P., Neglia, S., Gargiulo, A., Arcamone, N., Gargiulo, G., Varricchio, E., 2011. Immunohistochemical and immunochemical characterization of the distribution of leptin-like proteins in the gastroenteric tract of two teleosts (Dicentrarchus labrax and Carassius auratus L.) with different feeding habits. Microsc Res Tech. 74(8), 714-9.

Rosa Salva, O., Sovrano, V.A., Vallortigara, G. 2014. What can fish brains tell us about visual perception? Front Neural Circuits. 8:119.

Santana, S., Rico, E.P., Burgos, J.S., 2012. Can zebrafish be used as animal model to study Alzheimer's disease? Am J Neurodegener Dis. 1(1), 32-48.

Santoriello, C., Zon, L.I., 2012. Hooked! Modeling human disease in zebrafish. J Clin Invest. 122(7), 2337-43.

Satoh, N., Egami, N., 1972. Sex differentiation of germ cells in the teleost, Oryzias latipes, during normal embryonic development. J. Embryol. Exp. Morphol. 28, 385–95.

Schartl, M., 2014. Beyond the zebrafish: diverse fish species for modeling human disease. Dis Model Mech. 7(2),181-92.

Schmidt, R., Beil, T., Strähle, U., Rastegar, S., 2014. Stab wound injury of the zebrafish adult telencephalon: a method to investigate vertebrate brain neurogenesis and regeneration. J Vis Exp. 90,e51753.

Schmidt, R., Strähle, U., Scholpp, S., 2013. Neurogenesis in zebrafish - from embryo to adult. Neural Dev. 8, 3.

Sehnert, A.J., Huq, A., Weinstein, B.M., Walker, C., Fishman, M., Stainier, D.Y., 2002. Cardiac troponin T is essential in sarcomere assembly and cardiac contractility. Nat Genet. 31, 106–110.

Shanthanagouda, A.H., Guo, B.S., Ye, R.R., Chao, L., Chiang, M.W., Singaram, G., Cheung, N.K., Zhang, G., Au, D.W., 2014. Japanese medaka: a non-mammalian vertebrate model for studying sex and age-related bone metabolism in vivo. PLoS One. 9(2), e88165.

Sibbing, F.A., Uribe, R., 1985. Regional specializations in the oropharyngeal wall and food processing in the carp (Cyprinus carpio L.). Neth.J. Zool.35, 377–422.

Sidow, A., 1996. Gen(om)e duplications in the evolution of early vertebrates. Curr Opin Genet Dev. 6(6), 715-22.

Sollid, J., Nilsson, G.E., 2006. Plasticity of respiratory structures--adaptive remodeling of fish gills induced by ambient oxygen and temperature. Respir Physiol Neurobiol. 154(1-2):241-51.

Solnica-Krezel, L., Driever, W., 1994. Microtubule arrays of the zebrafish yolk cell: organization and function during epiboly. Development 120(9), 2443-55.

Sonawane, M., Carpio, Y., Geisler, R., Schwarz, H., Maischein, H.M., Nuesslein-Volhard, C., 2005. Zebrafish penner/lethal giant larvae 2 functions in hemidesmosome formation, maintenance of cellular morphology and growth regulation in the developing basal epidermis. Development 132(14), 3255-65.

Stacey, N., Chojnacki, A., Narayanan, A., Cole, T., Murphy, C., 2003. Hormonally derived sex pheromones in fish: exogenous cues and signals from gonad to brain. Can J Physiol Pharmacol. 81(4), 329-41.

Strähle, U., Scholz, S., Geisler, R., Greiner, P., Hollert, H., Rastegar, S., Schumacher, A., Selderslaghs, I., Weiss, C., Witters, H., Braunbeck, T., 2012. Zebrafish embryos as an alternative to animal experiments--a commentary on the definition of the onset of protected life stages in animal welfare regulations. Reprod Toxicol. 33(2), 128-32.

Suzuki, T., Nagano, H., Kobayashi, T., Ueno, K. 2005. Seasonal changes in the number of larvae and juveniles of crucian carps in the reed zone of Lake Biwa based on (sub) species identification using RAPD markers. Nippon Suisan Gakkaishi 71: 10-15

Taniguchi,K., Taniguchi, K., 2014. Phylogenic Studies on the Olfactory System in Vertebrates. J Vet Med Sci. 76(6), 781–788.

Terzibasi Tozzini, E., Lefrançois, C., Domenici, P., Hartmann, N., Graf, M., Cellerino, A., 2009. Effects of dietary restriction on mortality and age-related phenotypes in the short-lived fish Nothobranchius furzeri. Aging Cell, 8,88-99.

To, T.T., Witten, P.E., Renn, J., Bhattacharya, D., Huysseune, A., Winkler, C. 2012. Rankl-induced osteoclastogenesis leads to loss of mineralization in a medaka osteoporosis model. Development 139(1), 141-50.

Tohya, S., Mochizuki, A., Iwasa, Y., 2003. Difference in the retinal cone mosaic pattern between zebrafish and medaka: cell-rearrangement model. J Theor Biol. 221(2), 289-300.

Tomita, H., 1975. Medaka (Killifish): Biology and Strains (ed. Yamamoto, T.) 251–272 Keigaku Publishing Co., Tokyo, Japan.

Tozzini, E.T., Baumgart, M., Battistoni, G., Cellerino, A., 2012. Adult neurogenesis in the short-lived teleost Nothobranchius furzeri: localization of neurogenic niches, molecular characterization and effects of aging. Aging Cell. 11(2), 241-51.

Tsai, H.Y., Chang, M., Liu, S.C., Abe, G., Ota, K.G., 2013. Embryonic development of goldfish (Carassius auratus): a model for the study of evolutionary change in developmental mechanisms by artificial selection. Dev Dyn. 242(11), 1262-83.

Unniappan, S., Lin, X., Cervini, L., Rivier, J., Kaiya, H., Kangawa, K., Peter, R.E., 2002. Goldfish ghrelin: molecular characterization of the complementary deoxyribonucleic acid, partial gene structure and evidence for its stimulatory role in food intake. Endocrinology. 143(10):4143-6.

Valenzano, D.R., Terzibasi Tozzini, E., Cattaneo, A., Domenici, L., Cellerino, A., 2006. Temperature affects longevity and age-related locomotor and cognitive decay in the short-lived fish Nothobranchius furzeri. Aging Cell 5,275-278.

Valenzano, D.R., Cellerino, A., 2006. Resveratrol and the pharmacology of aging a new vertebrate model to validate an old molecule cell cycle 5:10, 1027-1032, Landes Bioscience.

Valenzano, D.R., Kirschner, J., Kamber, R.A., Zhang, E., Weber, D., Cellerino, A., Englert, C., Platzer, M., Reichwald, K., Brunet, A. 2009. Mapping loci associated with tail color and sex determination in the short-lived fish Nothobranchius furzeri. Genetics. 183(4):1385-95.

Valenzano, D.R., Sharp, S., Brunet, A., 2011. Transposon-Mediated Transgenesis in the Short-Lived African Killifish Nothobranchius furzeri, a Vertebrate Model for Aging. G3 (Bethesda). 1(7), 531-8.

van Raamsdonk, W., van't Veer, L., Veeken, K., Heyting, C., Pool, C.W., 1982. Differentiation of muscle fiber types in the teleost Brachydanio rerio, the zebrafish. Posthatching development. Anat Embryol (Berl). 164(1), 51-62.

Vandepoele, K., De Vos, W., Taylor, J.S., Meyer, A., Van de Peer, Y., 2004. Major events in the genome evolution of vertebrates: paranome age and size differ considerably between ray-finned fishes and land vertebrates. Proc Natl Acad Sci U S A. 101(6), 1638-43.

Velma, V., Tchounwou, P.B., 2011. Hexavalent chromium-induced multiple biomarker responses in liver and kidney of goldfish, Carassius auratus. Environ Toxicol. 26(6):649-56.

Videler, J. J., 1993. Fish Swimming. London: Chapman and Hall.

Volff, J.N., 2005. Genome evolution and biodiversity in teleost fish. Heredity (Edinb). 94(3):280-94.

Volkoff, H., Peter, R.E., 2006. Feeding behavior of fish and its control. Zebrafish. 3(2), 131-40.

von Hofsten, J., Olsson, P.E., 2005. Zebrafish sex determination and differentiation: involvement of FTZ-F1 genes. Reprod Biol Endocrinol. 3:63.

Wada, H., Shimada, A., Fukamachi, S., Naruse, K., Shima, A., 1998. Sex-Linked Inheritance of the lf Locus in the Medaka Fish (Oryzias latipes). Zoolog Sci. 15(1), 123-6.

Wallace, K.N., Akhter, S., Smith, E.M., Lorent, K., Pack, M., 2005. Intestinal growth and differentiation in zebrafish. Mech Dev 122, 157-173.

Wang, Z., Du, J., Lam, S.H., Mathavan, S., Matsudaira, P., Gong, Z., 2010. Morphological and molecular evidence for functional organization along the rostrocaudal axis of the adult zebrafish intestine. BMC Genomics 11, 392.

Waterman, R.E., 1969. Development of the lateral musculature in the teleost, Brachydanio rerio: a fine structural study. Am. J. Anat. 125, 457-93.

White, R., Rose, K., Zon, L., 2013. Zebrafish cancer: the state of the art and the path forward. Nat Rev Cancer. 13(9):624-36.

Whitfield, T.T., 2002. Zebrafish as a model for hearing and deafness. J Neurobiol. 53(2), 157-71.

Wittbrodt, J., Shima, A., Schartl, M., 2002. Medaka--a model organism from the far East. Nat Rev Genet. 3(1):53-64.

Witten, P.E., Huysseune, A., 2009. A comparative view on mechanisms and functions of skeletal remodelling in teleost fish, with special emphasis on osteoclasts and their function. Biol Rev Camb Philos Soc. 84(2), 315-46.

Wong, A.O., Chuk, M.C., Chan, H.C., Lee, E.K., 2007. Mechanisms for gonadotropin-releasing hormone potentiation of growth hormone rebound following norepinephrine inhibition in goldfish pituitary cells. Am J Physiol Endocrinol Metab. 292(1), E203-14.

Wullimann, M.F., Rupp, B., Reichert, H., 1996. Neuroanatomy of the Zebrafish Brain. A Topological Atlas. ISBN: 978-3-0348-9852-2.

Xu, X., Meiler, S.E., Zhong, T.P., Mohideen, M., Crossley, D.A., Burggren, W.W., Fishman, M.C., 2002.Cardiomyopathy in zebrafish due to mutation in an alternatively spliced exon of titin. Nat Genet. 30, 205–209.

Yamaha, E., Mizuno, T., Matsushita, K., Hasebe, Y. 1999. Developmental staging in goldfish during the pre-gastrula stage Nippon Suisan Gakkaishi, 65:709–717.

Yamaha, E., Usui, K., Onozato, H., Hamada K., 1986. A method for dechorionation in goldfish, Carassius auratus Bull. Jpn. Soc. Sci. Fish, 52:291–298.

Yamamoto, G., Takada, M., Iguchi, K., Nishida, M., 2010. Genetic constitution and phylogenetic relationships of Japanese crucian carps (Carassius). Ichthyological Research 57: 215-222.

Yamamoto, T., 1975. Medaka (Killifish): Biology and Strains. Keigaku Publishing Co., Tokyo.

Yamamoto, T., Kajishima, T., 1968. Sex hormone induction of sex reversal in the goldfish and evidence for male heterogamity. J Exp Zool. 168(2):215-21.

Zachar, P.C., Jonz, M.G., 2012. Neuroepithelial cells of the gill and their role in oxygen sensing. Respir Physiol Neurobiol. 184(3):301-8.

Zapata, A., 1979. Ultrastructural study of the teleost fish kidney. Dev. Comp. Immunol. 3, 55–65.

Zhao, L., Zhao, X., Tian, T., Lu, Q., Skrbo-Larssen, N., Wu, D., Kuang, Z., Zheng, X., Han, Y., Yang, S., Zhang, C., Meng, A., 2008. Heartspecific isoform of tropomyosin4 is essential for heartbeat in zebrafish embryos. Cardiovasc Res. 80, 200–208.

Zwollo P., Cole S., Bromage E., Kaattari S., 2005. B cell heterogeneity in the teleost kidney: evidence for a maturation gradient from anterior to posterior kidney. Journal of Immunology, 174(11), 6608–6616.

Zupanc, G.K., Hinsch, K., Gage, F.H., 2005. Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. J Comp Neurol. 488(3), 290-319.

http://www.genomesize.com

http://www.ensembl.org/Oryzias_latipes/Info/Index

http://www.zebrafishbrain.org/