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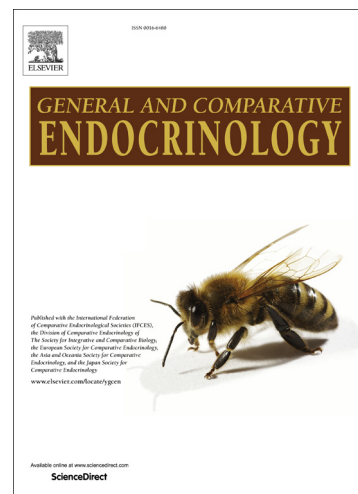
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**Why Goldfish? Merits and Challenges in Employing Goldfish as a Model
Organism in Comparative Endocrinology Research**

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HIGHLIGHTS

- The use of goldfish in comparative endocrinology research is rapidly growing.
- Goldfish offers several advantages as an animal model for comparative endocrinology.
- A great number of scientific techniques have been validated in goldfish.

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ABSTRACT

Goldfish has been used as an unconventional model organism to study a number of biological processes. For example, goldfish is a well-characterized and widely used model in comparative endocrinology, especially in neuroendocrinology. Several decades of research has established and validated a number of tools to study hormones in goldfish. The detailed brain atlas of goldfish, together with the stereotaxic apparatus is an invaluable tool for the neuroanatomic localization and central administration of endocrine factors. *In vitro* techniques, such as organ and primary cell cultures, have been developed using goldfish. *In vivo* approaches using goldfish were used to measure endogenous hormonal milieu, feeding, behaviour and stress. While there are many benefits in using goldfish as a model organism in research, there are also challenges associated with it. One example is its tetraploid genome that results in the existence of multiple isoforms of endocrine factors. The presence of extra endogenous forms of peptides and its receptors adds further complexity to the already redundant multifactorial endocrine milieu. This review will attempt to discuss the importance of goldfish as a model organism in comparative endocrinology. It will highlight some of the merits and challenges in employing goldfish as an animal model for hormone research in the post-genomic era.

Keywords: goldfish, model organisms, comparative endocrinology, neuroendocrinology, advantages, tools, techniques.

1. Introduction

Fishes represent the oldest, largest and most diverse class of vertebrates, comprising around 48% of the known member species of the subphylum Vertebrata. They live in a wide variety of habitats; from fresh to salt water, from cold polar seas to warm tropical reefs, and from shallow surface waters to the intense pressures of the ocean depths (Helfman et al., 2009). This ability to adapt to a wide variety of environments together with their evolutionary position relative to other vertebrates make them ideal for studying both organismic and molecular evolution. A number of other characteristics make fish an excellent experimental model in other research areas, including embryology, neuroendocrine signalling, neurobiology, endocrinology and environmental biology, among others. In addition, fish share many anatomical and physiological characteristics with mammals, including humans, which make them an important complement to mammalian models of disease. Ease to handle, commercial availability and relative low costs of rearing and maintenance are turning fish into the research model chosen by a growing number of researchers. Due to these reasons, and to a large extent due to the rapid expansion of the fish farming industry, the use of fish as research animal model has significantly increased worldwide over recent decades. While not a commercial species reared by aquaculture industry, the goldfish (*Carassius auratus*) found its solid place in comparative endocrinology, and helped reveal myriads of facts about the endocrine system. The use of this unconventional model also raises a routine question: “Why goldfish?”, or “Why are you using goldfish in your research?”. These questions are routinely posed to in-training students and established researchers using goldfish. The answer to this question is very complex. The intention behind this review is to provide a one-stop article to learn more about goldfish as an experimental model. Earlier, Popesku et al. (2008) provided an elegant review that solely focused on

goldfish as a model for studying neuroendocrine signalling. Our aim is to provide a comprehensive article enabling the readers to become more knowledgeable about goldfish, and answer the above-mentioned questions. It could be used as a reference tool for all goldfish users. In addition to that, this review would offer a wider aspect on the importance of goldfish being considered as an important model for comparative endocrinology research.

Goldfish is a freshwater fish that belongs to the family Cyprinidae of order Cypriniformes (**Table 1**), native to East Asia. As a member of the Cyprinidae family, goldfish is related to important ecological and genetic models, including zebrafish, and to carp, an economically important cultured species. Selective breeding over centuries has led to numerous breeds of goldfish, which vary in size, body shape, colouration, and fin and eye configuration (Smartt, 2001; Walker and Johansen, 1977). These breeds include the common and comet varieties used for research, and other fancy varieties such as the black telescope, fantail, bubble eye, celestial eye and butterfly tail goldfish, to name a few. There are several theories available about the origin and evolution of goldfish breeds. It appears that China, Japan and the USA contributed heavily to goldfish breeding. However, authentic information to safely conclude on the origin and breeding timeline of currently available strains of goldfish is missing. Goldfish generally reach 15–20 cm, weigh 100 – 300 g (Szczerbowski, 2001) and live typically for 6-7 years. Their natural habitat includes the quiet backwaters of streams and pools, especially those with submerged aquatic vegetation (Hensley and Courtenay, 1980). Goldfish prefers pH levels of 5.5–7.0 (Szczerbowski, 2001), although pH tolerance levels between 5.4-10.5 have been reported. It is also tolerant of high levels of turbidity (Wallen, 1951), temperature fluctuations (Spotila et al., 1979), and low levels of dissolved oxygen (Walker and Johansen, 1977). Adults thrive equally well in salinities

between 0–6 ppt (Canagartnam, 1959), and can survive water temperatures between 0–41 °C (Carlander, 1969).

The use of goldfish in scientific works goes as far back as 1901, when W. L. Underwood first described the role of goldfish in devouring mosquito larvae (Underwood, 1901). Since then, the use of goldfish in research has been increasing considerably over the years, and a steep increase in using goldfish was noted between 1960 and 1980 (**Figure 1A**). A search in PubMed using the term “goldfish” resulted in more than six thousand publications, arising from all parts of the globe (**Figure 1B**). Most of these articles describe endocrinology and how the interactions between brain and peripheral organs regulate growth, appetite behaviour, energy homeostasis, metabolism, reproduction, gonadal physiology and stress response. However, goldfish also serves as useful model organism in cell biology, immunology, toxicology, molecular evolution and comparative genomics, neurobiology, olfaction, vision and taste. Behavioural aspects such as locomotor activity have also been extensively studied using goldfish as animal model, and recently diverse behavioural tests for anxiety, fear and stress were proposed using this teleost species (Maximino et al., 2015).

One of the main and simpler advantages of using goldfish is its capability to thrive under laboratory conditions and ease in handling and sample collection. In this regard, its availability in desirable sizes compared to other commonly used teleosts, including salmon and zebrafish, makes it easier for maintenance and suitable for the collection of considerable amounts of blood and other tissues to perform *in vivo*, and *in vitro* biochemical and molecular analyses. In addition, multiple, sequential blood sampling is also possible with goldfish. Several decades of research has established and validated a number of research tools for use in goldfish. For example, the brain atlas and the stereotaxic apparatus are invaluable tools for the neuroanatomic localization of

hormones and its receptors, and for the central administration of peptides (Peter and Gill, 1975). Goldfish is equally suitable for both *in vivo* and *in vitro* studies. This include primary cell and tissue cultures and organ baths systems, which are well-established for goldfish and have offered tremendous knowledge on the physiology of central and peripheral organs (Grey and Chang, 2011; Kobayashi et al., 2014; Tinoco et al., 2015; Velarde et al., 2010; Volkoff, 2014). Additionally, different techniques are available to study goldfish behaviour, including locomotor activity (Azpeleta et al., 2010; Nakamachi et al., 2014; Vera et al., 2007; Vivas et al., 2011; Yahashi et al., 2012). This review will attempt to discuss the importance of goldfish as a model organism in integrative physiology, neuroendocrinology and comparative endocrinology. We will approach this by reporting the most important characteristics of the goldfish. A separate section on the use of goldfish for behavioural studies is also included.

2. Anatomy

The goldfish body shape is relatively short and compact, and possesses five sets of fins following the pattern of most of the Cyprinidae family: dorsal fin on the back, caudal (tail) fin, anal fin, ventral or pelvic fins and pectoral fins (**Figure 2A-C**). The scales are large, uniform in both size and shape, and overlap in a regular pattern. The eyes are situated on both sides of the head with little movement possible; indeed, goldfish are considered short-sighted, relying on other senses to find food and warn of danger. On each side of the head, a hard bony flap (operculum), open in the back for the release of water, covers and protects the gills. Goldfish have a continuous lateral line canal system that consists of supraorbital, infraorbital, operculomandibular and supratemporal commissural canals on the head, and a trunk canal extending the length

of the trunk. The goldfish lateral line is extremely sensitive, and can detect motion, current, pressure, temperature and sound.

Internal goldfish anatomy shows all organs found in vertebrates, as can be seen in **Figures 2 A-C**. The brain is a highly complex and organized system divided into discrete areas involved in the regulation of the different physiological and behavioural functions. The primary olfactory structures, the paired olfactory bulbs, are prominent, and the lateral and medial olfactory tracts (which contain the secondary olfactory projections originating in the olfactory bulbs) form relatively long stalks (Rupp et al., 1996). The rest of the telencephalon, the optic tectum, the hypothalamus and the cerebellum follow the same pattern as in other related fish, including zebrafish. However, in contrast to that species, the paired vagal lobes in goldfish form a large, separate brain part caudal to the cerebellum (Rupp et al., 1996).

Below the brain lies the pituitary gland or hypophysis. Morphologically, the pituitary gland is typically divided in two regions, the adenohipophysis and the neurohypophysis. Teleost adenohipophysis can be likewise divided in three conserved lobes: the proximal *pars distalis*, the rostral *pars distalis* and the neurointermediate lobe. The proximal *pars distalis* contains gonadotrophs and somatotrophs. The most abundant class of cells in the rostral *pars distalis* are lactotrophs, but also thyrotrophs and corticotrophs are present at the border between the rostral and proximal *pars distalis*. The neurointermediate lobe contains the *pars intermedia*, which is innervated by the homologue of the caudal neurohypophysis. The anterior neurohypophysis (homologous to the median eminence) innervates the rostral and proximal *pars distalis* (Kaul and Vollrath, 1974). The teleostean pituitary gland possesses important differences in relation to mammalian pituitaries. First, the distribution of endocrine cells within the pituitary is highly regionalized in teleosts compared to mammals, in which a

dispersed organization of endocrine cells is found. Second, the teleostean pituitary lacks a functional hypothalamo-hypophyseal blood portal system and instead its endocrine cells are directly innervated. Anglade and coworkers (1993) were able to precisely determine the entire hypophysiotrophic inputs to the pituitary in fish using neuronal tract-tracing techniques. In mammals and other tetrapods, the presence of the hypothalamic-hypophyseal blood portal system makes it more complicated due to the multiple neurotransmitter and neuropeptidergic inputs converging at the median eminence (Popesku et al., 2008).

The goldfish heart is constituted by the typical four chambers, i.e. sinus venous, atrium, ventricle and bulbus arteriosus, and also contains an atrio-ventricular region and a muscularized conus arteriosus supporting the conus valves (Garofalo et al., 2012). Various studies have been performed to review the internal organization of fish heart chambers and their cardiac activity (Santer 1985., Satchell 1991., Burggren et al., 1997). Based on their external shape, fish heart ventricle has been classified into three major categories, i.e. sac-like, identified by its round and blunt apex; tubular, characterized by cylindrical structure; and pyramidal, with a triangular base (Santer, 1985). However, according to the myocardial arrangement, four ventricle types can be distinguished (Farrell and Jones, 1982; Tota et al., 1983). In Type-I, the myocardial arrangement of the ventricle is avascular and trabeculated. Type-II has a ventricular myocardium that has both outer vascularized and inner *spongiosa*. The Type-III has ventricular vessels present both at outer vascularized and inner *spongiosa*. Finally, Type-IV has ventricles that are characterized by its vascularized myocardium. Goldfish possesses a Type-II heart ventricle, which has a thin vascularized layer that extends towards the avascular spongy heart. With respect to ventricular arrangement, goldfish has a sac shaped heart, when compared to zebrafish which has a more pyramidal heart (Grivas et al., 2014).

The abdominal cavity of goldfish is mainly filled with the gastrointestinal tract, an elongated, relatively undifferentiated long tube. The most notable feature of this tract is the lack of a stomach comparable to that of other vertebrates. Instead, an enlargement of the intestine, known as the intestinal bulb, replaces stomach. The digestive tract could thus be divided into mouth, buccal cavity, pharynx, oesophagus, intestinal bulb, intestine, and rectum. The different regions are not very well demarcated, but can be distinguished by changes in the character of the epithelial lining, or by valve-like structures at the junctions between the adjoining regions (Sarbah, 1951). The esophagus is a short tubular structure, adjacent to the pericardial cavity, upholstered by a mucous membrane characterized by prominent longitudinal folds. Although no well-marked indication of the oesophagus passage into the intestinal bulb can be seen externally, a valve-like structure separate each other internally (McVay and Kaan, 1940; Sarbah, 1951). The intestinal bulb is a thickened straight tube, capable of great expansion, which extends into the posterior end of the abdominal cavity. This portion is larger and straight at its anterior end, and narrows gradually until it merges into the intestine proper, which occupies the ventral portion of the abdominal cavity (McVay and Kaan, 1940). The folds of the intestinal mucous membrane present a zig-zag pattern, which in the anterior region is characterized by straight lines and angles, while in the posterior portion is produced by a convoluted lining (Sarbah, 1951).

The gastrointestinal tract is in tight relationship with the liver, the spleen and part of the adipose tissue. The goldfish liver is diffused and formed by narrow right and left hepatic lobes, extending on each side of the intestinal bulb. The two lobes join at their anterior and posterior ends to form the anterior median and posterior median hepatic lobes. The anterior median lobe is attached to the posterior face of the transverse septum separating the pericardial and abdominal cavities, while the posterior median

lobe is extended beyond the limit of the bulb. Embedded in the parenchyma of the liver lie the rudimentary pancreas (Sarbahe, 1951), thus forming a structure that some authors have referred to as a hepatopancreas. Two main differences were found between the liver of goldfish and other vertebrates. The first one is that goldfish liver is composed mainly by hepatic plates that are two cells in thickness, while the hepatic plates of several other non-fish species were primarily one-cell thick (Elias and Bengelsdorf, 1952). This tissue in goldfish does not show the typical lobulation or arrangement into plates found in the mammalian liver, but instead goldfish liver cells are closely packed between the capillaries (sinusoids) which are irregularly distributed (Yamamoto, 1965). The second difference lies in the occurrence of true intracellular bile canaliculi (tubes that collect bile secreted by hepatocytes) in goldfish, which consist entirely of intracellular channels inside the liver cells. However, no intercellular bile canaliculi are formed in goldfish liver cells (Yamamoto, 1965).

The spleen, consists primarily of red pulp and is normally an elongated organ lying inside the serosal lining of the intestine. It acts primarily as a blood filter, expressing high levels of thrombopoietin (TPO) which play an important role in regulation of platelet production, progenitor cell proliferation and differentiation of megakaryocytes contributing to immune system (Katakura et al., 2015). The fish adipose tissue differs in their localization and their morphological characteristics. Although goldfish is not characterized by having large amounts of adipose tissue, fat depots can be found in the abdominal cavity around the digestive tract (visceral or mesenteric fat) or all around the body of the fish (subcutaneous fat) (Weil et al., 2013).

The goldfish kidney, like in many other fish, is a distinctly bilobed organ consisting of a cranial and a caudal kidney. The cranial or head kidney is an immune and endocrine organ containing hematopoietic and endocrine tissue, which is placed

around the posterior cardinal vein (Sampour, 2008). The hematopoietic tissue consists of cells that play an important role in producing lymphoid (B- and T-lymphocytes) and myeloid (phagocytic) cells, which forms an integral analogue of the mammalian bone marrow. The endocrine tissue is composed of interrenal cells (interrenal tissue; cortical) producing cortisol and chromaffin cells (medullary) producing catecholamines, making it the functional analogue of the mammalian adrenal gland (Verburg-Van Kemenade et al., 2009). For its part, the caudal kidney is formed by nephrons surrounded by hematopoietic and lymphoid tissue dispersed throughout the organ. Nephrons typically have a well-vascularized glomerulus, a ciliated neck segment, two proximal segments, a ciliated intermediate segment, a distal segment, and a collecting duct system (Reimschuessel, 2001).

Teleosts possess loosely organized thyroids that may occur in locations other than in the throat or lower jaw. In the case of the goldfish, apart from containing a pharyngeal thyroid, it has normal thyroid follicles in the head kidney (Chavin, 1956). The epithelial heights of such follicles were found to respond to physiological alterations similar to those of the normally located thyroid. Indeed, it has been demonstrated that the goldfish head kidney thyroid accumulates about twice as much radioiodine as does the pharyngeal thyroid (Chavin and Bouwman, 1965).

Finally, the gonads of goldfish, like other teleosts, lack medullary tissue, thus corresponding only to the cortex of other vertebrates. Morphologically, male gonads (testes) are elongated paired organs attached to the dorsal body wall. A main sperm duct arises from the posterior mesodorsal surface of each elongated testis and leads to the urogenital papilla located between the rectum and the urinary ducts. The testes are composed of numerous lobules, which are separated from each other by a thin layer of connective tissue. Based on the arrangement and distribution of stem spermatogonia,

spermatogenesis in goldfish is classified as tubular (non-restricted), thereby producing free spermatozoa during spermatogenesis. Spermatogenesis occurs within the lobules, and then the sperm is liberated into the lobular lumen, which is continuous to the sperm duct (Nagahama, 1983). In females, the ovary consists of oogonia, oocytes and their surrounding follicle cells, supporting tissue or stroma, and vascular and nervous tissue. Goldfish ovary is asynchronous, which means that it contains oocytes at all stages of development. This type of ovary is frequent in fish such as goldfish, which spawn many times during a prolonged breeding season (Nagahama, 1983).

3. A system approach for the consideration of goldfish as a model organism

3.1. Brain

One of the main advantages the goldfish brain offers to researchers in terms of handling is its size, which makes it possible to carry out studies using both the whole brain as a single unit or to identify and dissect the different areas separately. The use of the goldfish brain and/or brain sections has led to important contributions to scientific knowledge. For example, the cDNA encoding some hormones and their receptors in goldfish, such as kisspeptins (Li et al., 2009) and neuromedin U (Maruyama et al., 2008), have been isolated from whole brain samples. Discrete areas of the goldfish brain have been subject to a large numbers of studies, mainly focused on the expression profile and regulation by different factors of transcripts of genes encoding neuroendocrine hormones or enzymes (Bertucci et al., 2016; Blanco et al., 2016a; Popesku et al., 2012; Volkoff, 2013). Expression studies using goldfish brain areas have also been carried out to study interactional aspects between the circadian and endocrine systems (Feliciano et al., 2011; Iigo et al., 1994; Nisembaum et al., 2014; Tinoco et al., 2014).

Goldfish brain sections have also been examined under tissue culture conditions, validating *in vitro* approaches that offer an important tool to be applied to studies in different fields. Examples include the study of neurophysiological and biochemical properties of a discrete brain area (Teyler et al., 1981), the activity of enzymes and its regulation by external factors (Hall et al., 1982), the modulation of respiratory activity (Côté et al., 2014), and the role of hormones (Volkoff, 2014). Some researchers have successfully developed a method for isolating and culturing specific brain cell types, such as astrocytes (Sivron et al., 1993), and microglia-like cells (Houalla and Levine, 2003). These approaches provide important knowledge on the maintenance of brain homeostasis, protection of neurons and control of neuronal proliferation.

The nuclear organization of the goldfish brain is described in the brain atlas (Peter and Gill, 1975) that offers an invaluable tool for neuroanatomical studies. This has made available the brain mapping of a large number of hormones, receptors, and enzymes in goldfish (Blanco et al., 2016b; Canosa et al., 2004; Cerdá-Reverter et al., 2003a; Giraldez-Perez et al., 2009; Kojima et al., 2010; Sánchez-Bretaño et al., 2015b, 2015c; Wang et al., 2000). Numerous works have also been focused on the neural connections existing among the different areas of the goldfish brain, and important and abundant information is available in the literature on the projections within the goldfish brain and the pathways involved in the different sensory systems and physiological actions (Ikenaga et al., 2002; Kato et al., 2012, 2011; Luque et al., 2008; Northcutt, 2006; Uezono et al., 2015).

Among the diverse types of neurons present in the goldfish brain, the Mauthner cells are a pair of big and easily identifiable neurons located in the hindbrain of most fish and amphibians (Zottoli and Faber, 2000). Due to its large size and prominent roles in the initiation of swimming, equilibrium maintenance and initiation of startle reflex in

goldfish, the Mauthner cell is one of the most studied vertebrate neurons and has provided an impetus for an extraordinary array of interdisciplinary studies focused on a single reticulospinal neuron. Indeed, many first discoveries in vertebrate neuroscience were based on Mauthner cells. Examples include the electronic transmission and neuronal gap junctions (Furshpan, 1964; Robertson et al., 1963), the remote dendrite inhibition (Diamond and Huxley, 1968), and the correlation of a single cell activity with a defined response in a free-swimming animal (Eaton et al., 1981; Zottoli, 1977). A significant part of the up-to-date knowledge on the functioning of Mauthner cells is derived from studies on goldfish, which has served as a model organism for studying this type of cell in terms of structure (Robertson et al., 1963), mechanism of action (Diamond and Roper, 1973; Faber and Korn, 1988; Furukawa, 1966; Hatta et al., 2001; Kono, 1970; Lin and Faber, 1988a, 1988b; Pereda et al., 1994), inputs/outputs (Fay, 1995; Gotow et al., 1990; Mirjany and Faber, 2011; Nissanov et al., 1990; Scott et al., 1994; Zottoli et al., 1987) and regulation (Cachope and Pereda, 2015).

The goldfish brain is a great model for intracerebroventricular (icv) injections to test the central effects of hormones and drugs. Several variants of this method of drug administration have been described. For instance, Peter and co-workers performed stereotaxic apparatus-based injections into the third ventricle after exposing the brain by cutting the roof of the skull along three sides of a square and folding the skull bone flap to one side (Narnaware et al., 2000). De Pedro and colleagues, instead, do not expose the brain and perform freehand injections directly through the central junction between the parietal and frontal bones (De Pedro et al., 1993). Some scientists, including the laboratories led by Volkoff (Hoskins et al., 2008), Matsuda (Matsuda et al., 2006a), Himick (Himick and Peter, 1994) and Unniappan (Gonzalez and Unniappan, 2010), placed the fish in a stereotaxic apparatus and, after exposing the brain, perform the

injections into the third ventricle according to coordinates taken from the stereotaxic atlas of the goldfish brain (Peter and Gill, 1975). ICV injections in goldfish was also used to study behavioral and neuroendocrine aspects in addition to food intake (Bernier et al., 2004; Cerdá-Reverter et al., 2003b; de Pedro et al., 2000; De Pedro et al., 1998; Gonzalez et al., 2010; Matsuda et al., 2006b; Nakamachi et al., 2006; Narnaware and Peter, 2001; Unniappan et al., 2004; Volkoff et al., 2003). The possibility of carrying out ICV injections allows the comparison between the effects of peptides directly injected into the brain *versus* injected peripherally by intraperitoneal (ip) injection, and so crossing the blood-brain barrier. Some additional examples include the study of the neuroendocrine regulation of food intake, in which goldfish served as a teleost fish model to compare and contrast information that was previously collected from mammals (Blanco et al., 2016a; Cerdá-Reverter and Peter, 2003; Gonzalez et al., 2010; Matsuda et al., 2009; Sánchez-Bretaña et al., 2015b; Tinoco et al., 2012).

Another important technique that has been used in goldfish brain is the recording of electrical responses. This technique has been employed, for instance, in several studies on the olfactory system of goldfish and its behavioural responsiveness to sex pheromones and food odours. In this sense, it has been demonstrated that the medial olfactory tract responds to pheromones and both the medial and lateral sub-tracts are in charge of the responses to food odours (Sorensen, 1996; Sorensen et al., 1991). Moreover, this type of studies have allowed scientists to determine that sex pheromones and bile steroids elicit relatively small responses in comparison to amino acids, which produce large responses. The results of such studies also suggest that vertebrate pheromones are discriminated by a distinctive subcomponent of the vertebrate olfactory system which is comprised of a relatively small number of olfactory neurons (Hanson et al., 1998).

The goldfish brain has also been the subject of numerous lesion studies. This type of studies has offered valuable seminal information on many physiological aspects of brain functioning in goldfish. For example, in the late 70's it has been reported that lesions of the lateral areas of the hypothalamus cause the cessation of operating feeding, thus suggesting these hypothalamic areas as key areas involved in the regulation of food intake (Peter, 1979; Roberts and Savage, 1978). The involvement of the olfactory tracts on feeding responses, as well as on sexual behaviour in goldfish, has also been suggested by transection of the different subdivisions of the olfactory tract (Stacey and Kyle, 1983). Lesion techniques have been also used to study the ultrastructure of specific areas of the brains, such as the olfactory epithelium (Hansen et al., 1999). In the recent past, studies using the ablation of different areas of the goldfish brain have demonstrated the specific involvement of the different areas in more behavioural aspects, such as taste aversion learning (Martín et al., 2011), memory (Broglia et al., 2010), spatial and temporal cognition and learning (Durán et al., 2010; Portavella et al., 2002; Saito and Watanabe, 2006; Vargas et al., 2006), emotional and motor response conditioning (Gómez et al., 2010; Rodríguez et al., 2005), and the shoaling behaviour (Shinozuka and Watanabe, 2004).

In addition to that, researchers have also studied the caudal neurosecretory system of goldfish. The caudal neurosecretory system of goldfish consists of complex secretory neurons that have been differentiated as large, medium and small Dahlgren cells (neurosecretory cells) leading to a neurohaemal tissue arranged as a neurosecretory releasing organ termed as urophysis (Cioni et al., 1998; Fridberg and Bern, 1968). In goldfish, small cells were localized in the spinal cord dorsal to urophysis, whereas medium-sized neurosecretory cells are located in the anterior part of urophysis, and the large sized cells are further anterior to the medium sized Dahlgren cells (Owada et al.,

1985). The neurosecretory cells/Dahlgren cells originate from the ependymal cells, which play an important role in development and regeneration of caudal system in goldfish. It has been shown that when a cut proximal to the urophysis is done in the neurosecretory tract, the neurohemal area regenerates at the site of transection, allowing the survival of the neurosecretory cells proximal to the cut. Additionally, it was reported that ependymal cells can transform into Dahlgren cells on regeneration after total extirpation of the caudal neurosecretory system in some teleostean species (Kobayashi et al., 1986). Apart from this important feature, the urophysis has been notably linked to osmoregulation and ionic regulation. In this regard, it has been shown that administration of urophysial extract results in an increase in the uptake of sodium by the gills of goldfish (Fridberg and Bern, 1968).

In the last decade, teleosts, including goldfish, have been employed to study regenerative processes within the brain. One particularly notable case in the completeness and fidelity of regeneration is the retinotectal system of the goldfish (Matsukawa et al., 2004). In this species, when the optic nerve was severed, all retinal ganglion cells survived, and after about a week, commenced to regenerate past the injury and began to invade the tectum. By 3–4 weeks, regenerating fibers encompassed the entire tectum. Using an *in vivo* imaging technique, Dawson and Meyer (2008) succeeded in analysing the dynamic behaviour and morphology of regenerating axons in the goldfish optic tectum. The dynamic behaviour of axons in systems that normally regenerate may provide clues for promoting regeneration in humans.

3.2. Pituitary

The pituitary gland of goldfish has been the subject of a wide variety of research studies. Goldfish pituitary has been also used to study the neuroendocrine regulation of fish growth (Azuma et al., 2013, 2012; Grey and Chang, 2009; Matsuda et al., 2008).

Moreover, since the discovery of gonadotropic hormones I and II (GTH-I and GTH-II, respectively) (Querat, 1994; Yoshiura et al., 1997) and its receptors (Yan et al., 1992), which are structurally similar to the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) systems from mammals, fish became a good model for comparative studies on the neuroendocrine regulation of reproduction. Both GTH-1 (FSH) and GTH-II (LH) are secreted from the pars distalis of goldfish. GTH-1 has been shown to stimulate gonadal steroidogenesis. However, the most relevant of these hormones seems to be GTH-2, which has been shown to stimulate gonadal steroidogenesis, gametogenesis and ovulation, thus being a key regulator of fertility (Peter et al., 1991; Trudeau, 1997). Goldfish has been extensively used to study the hypothalamus-pituitary-gonadal axis (Hontela and Peter, 1980), the regulation of gonadotropic hormones release (Chang et al., 1983; Trudeau, 1997; Wong et al., 2001) and the influence of environmental factors such as temperature (Fraser et al., 2002; Hontela and Peter, 1978; Sohn et al., 1998) and photoperiod (Hontela and Peter, 1978) on pituitary functions. Many of the studies mentioned above were performed *in vitro*, thanks to the relative big size of this tissue (compared to other fish species) and the possibility to isolate it properly and easily. A number of *in vitro* techniques have been validated for studies on goldfish pituitary. This ranges from the culture of pituitary fragments (Peng et al., 1993, 1990), to a dispersed pituitary cell system in static primary culture or in perfusion culture (Grey and Chang, 2009; Lee et al., 2000; Tanaka et al., 2009; Unniappan and Peter, 2004; Yunker et al., 2000). Since the pituitary fragments contain nerve endings containing pituitary regulatory hormones (e.g. the gonadotropin-releasing factor, GnRH), the use of dispersed pituitary cells is relatively more appealing to scientists. The establishment of pituitary cell/organ *in vitro* culture defined the

remarkable progress made in understanding the complex regulation of pituitary hormones in fish.

3.3. Gastrointestinal tract

The goldfish gastrointestinal tract has been the subject of large number of studies. Mainly two types of cells with endocrine functions are found within the intestinal epithelium of fish, including goldfish. These types of mucosal cells are open-type or columnar cells, and closed-type or goblet cells, wherein the former cell type is more abundant and characterized by having their base at the basal lamina and an apical process that reaches the lumen, and the latter cells are big and round, and located away from and not adjacent to the inner surface. In addition to an apical process, some endocrine cells also have basal processes that reach out and make contact with neighbouring cells, giving them a paracrine function (Holmgren et al., 1986). A great number of brain-gut peptides have been localised in goldfish endocrine cells. Examples include gastrin and cholecystokinin (CCK) (Himick and Peter, 1994; Kiliaan et al., 1992), ghrelin (Arcamone et al., 2009; Sánchez-Bretaña et al., 2015b), neurotensin (Kiliaan et al., 1992), somatostatin (Canosa et al., 2005), and peptide YY (Gonzalez and Unniappan, 2010b), to name of a few. Numerous studies involving these peptides have been performed in goldfish to determine their role in regulating feeding and other physiological processes. For instance, ghrelin is known to be a potent stimulator of food intake in this species (Unniappan et al., 2004), as well as an important enhancer of growth hormone release by pituitary (Unniappan and Peter, 2004). On the other hand, CCK has been shown to have an anorexigenic effect on goldfish after intracerebroventricular administration (Himick and Peter, 1994). It was also demonstrated that this hormone mediate the actions of leptin and some orexigen, such as neuropeptide Y (NPY) and orexin-A, in goldfish (Volkoff et al., 2003).

As in the case of the gut of other vertebrates, the goldfish gut, has the ability to receive food, process it physically and chemically, absorb nutrients and dispose off the wastes. The control of these functions relies on the enteric nervous system and substances released from the endocrine and paracrine cells of the gut mucosa, which affects mainly secretion and motility. In this regard, experiments on isolated strip preparations of intestine have shown that substances including CCK (Tinoco et al., 2015), substance P (Kitazawa et al., 2012) and neurotensin (Kitazawa et al., 2012) modify gut contractility by exerting an excitatory or inhibitory effect on the smooth muscle. Furthermore, using the Ussing-type chamber technique, the goldfish intestine has been employed to study the effects of this type of substances on electrophysiological parameters of the gut, such as the ion selectivity of the intestinal epithelium (Kiliaan et al., 1992). In addition to that, goldfish intestine secretes serum amyloid A (SAA), which plays an important role in stimulating pro- and anti-inflammatory cytokine expression (IL-10, TGF- β) in monocytes and macrophage development. Also, recombinant SAA have been shown to downregulate nitric-oxide production and induced chemotaxis of neutrophils and macrophages in goldfish (Kovacevic and Belosevic, 2015). Goldfish intestine is an important site of interferon gamma (IFN γ) release, and plays an important role in the regulation of proinflammatory cytokines, enhanced respiratory burst responses, increased phagocytic and nitric oxide production in goldfish macrophages (Grayfer and Belosevic, 2009).

3.4. Liver or hepatopancreas

In fish, as in other vertebrates, the liver is the key organ controlling many functions in anabolism and catabolism, and the fish liver also plays an important role in vitellogenesis. This latter process has been studied in the goldfish liver to test the hypothesis that it might be influenced by thyroid hormones. In this respect, it was

reported that the thyroid hormone triiodothyronine (T3) increases vitellogenin in cultured hepatocytes from female, but not male, goldfish (Nelson and Habibi, 2016). Since fish are more susceptible to environmental variations than mammals, their liver is a very useful model to study interactions between environmental factors and hepatic structures and its functions (Bruslé and Anadon, 1996). Very recently, results from *in vitro* studies on tissue culture have identified the liver of goldfish as an important component of the circadian system of this species (Sánchez-Bretaña et al., 2016, 2015a).

3.5. Adipose tissue

Adipose tissue is an important site for the regulation of steroid homeostasis in fishes. Indeed, studies performed in another teleost species, the largemouth bass, have shown that an excess in mesenteric adipose tissue leads to an alteration of the reproductive cycle, marked by reduced testosterone and estradiol levels during early recrudescence, and a delay in spawning (Rosenblum et al., 1994). Using goldfish mesenteric adipose tissue culture, it has been suggested that this tissue is capable of converting testosterone to several metabolites, including estrogen (Moore et al., 2002).

3.6. Head Kidney

The goldfish head kidney offers a tool to study stress and immunity. However, only limited number of studies using this tissue was reported. Among them, and considering the endocrine portion of the head kidney, some studies are available on the role of cortisol (Bernier et al., 1999, 2004, Chasiotis and Kelly, 2011, 2012; Fryer et al., 1984; Wang and Belosevic, 1995) and the control of its release (Azpeleta et al., 2010; Eslamloo et al., 2014; Fryer et al., 1983; Fryer and Leung, 1982; Jung et al., 2016; Vera et al., 2007; Woo et al., 1985), most of them based on the analysis of the effects of cortisol injections and/or the determination of circulating cortisol levels. However,

probably due to its anatomy, very few studies using the goldfish head kidney *in vivo* (Kobayashi et al., 2011; Lim et al., 2013) or *in vitro* (Kobayashi et al., 2014) have been conducted. Studies related to fish immunology using goldfish are extensive. Macrophage colony stimulating factor (CSF-1) is considered to be an important factor in macrophage development. Goldfish CSF-1 functionally appears to act similar to the mammalian CSF-1 in the regulation of monocytes and macrophages. In addition to that, infusion of recombinant CSF-1 to goldfish macrophage cultures resulted in their stabilization and in an increase in the longevity of functional macrophage production, thereby leading to a stimulated respiratory burst and production of nitric oxide response upon activation with goldfish recombinant TNF- α (Hanington et al., 2009; Grayfer et al., 2009). CSF-1 has been identified as a key regulator of pro-inflammatory and phagocytic responses in goldfish (Grayfer et al., 2009). These studies have paved way in the use of goldfish as a model organism to study immunology and immune-endocrine interactions in fish.

3.7. Caudal Kidney

The caudal kidney, for its part, has been used as a model for studying renal injuries, especially those induced by exposure to toxicants (Munkittrick et al., 1985; Reimschuessel et al., 1990; Reimschuessel and Williams, 1995). However, its more remarkable characteristic is its ability to repair injured nephrons. This response occurs in multiple fish, including goldfish, zebrafish, catfish, trout and tilapia, placing the fish kidney as a unique model for investigating renal injury, repair, and development. The repair is marked by replacing the injured cells with new epithelial cells, restoring tubule integrity (Reimschuessel, 2001). In addition, fish have the ability to respond to renal injury by *de novo* nephron development (Reimschuessel et al., 1990; Reimschuessel and

Williams, 1995; Salice et al., 2001), a response that is not observed in mammals. This nephrogenic response, apart from providing a more abundant source of developing renal tissue compared with fetal mammalian kidneys, may also help to identify novel genes involved in nephrogenesis, information that could eventually be used to develop alternative renal replacement therapies.

3.8. Gonads

A large focus on fish endocrinology is on reproduction, and goldfish has been widely used to investigate the hormonal regulation of reproduction. Goldfish possesses an XX–XY sex determination system (Yamamoto and Kajishima, 1968), but it has been demonstrated that it is possible to manipulate its phenotypic sex by temperature (Goto-Kazeto et al., 2006). In fact, temperature and photoperiod seem to be the major regulators of gametogenesis in goldfish reproduction. In temperate climates in the northern hemisphere, cyprinid fish spawn in spring when the water temperature reached between 18–21°C (Razani et al., 1987). A great number of the studies using goldfish gonads are focused on the influence of gonadotrophins and/or other pituitary and hypothalamic hormones on gonadal development and function of both females and males (Ge et al., 1993; Habibi et al., 1989; Peter and Crim, 1978; Trudeau, 1997). The influence of season and environmental factors on gonadal growth and function in goldfish has also been widely studied (Delahunty and de Vlaming, 1980; Spieler et al., 1977). In the last decade, there has been increasing evidence that thyroid hormones influence reproduction in vertebrates. The goldfish has served as a valid model to offer information on the mechanisms by which this regulation occurs (Nelson et al., 2010, 2011). Finally, goldfish is commonly used to test the role of some water contaminants as potential neuroendocrine disruptors, and their influence on gonadal function (Nadzialek et al., 2008; Spanò et al., 2004).

Ovary

The relationship between body weight and the weights of ovary, liver and visceral lipid stores in goldfish was examined at different times of the year (Delahunty and de Vlaming, 1980). The authors found monthly differences in the ovary weight relative to body weight, with a peak in April. Also, Gillet and coworkers (1978) have shown that temperature might have a direct effect on gonads by modifying ovarian responsiveness to GtH. Ovarian maturation in goldfish has also been studied in relation to photoperiod, and it has been observed that an increase in light exposure stimulates ovarian maturation although the efficacy of this increase is subject to the time of the year (Fenwick, 1970). In terms of the role of thyroid hormones on reproduction, it has been shown that the three estrogen receptor subtypes, as well as the expression of gonadal aromatase, are downregulated by the thyroid hormone T3 in the ovary of goldfish (Nelson et al., 2010). Furthermore, female goldfish has been used to test the potential role of certain substances as endocrine disruptors, measuring the effect of these compounds on the ovarian production of estradiol, 11-ketotestosterone and aromatase (Nadzialek et al., 2008).

Testis

The goldfish testis has served as a model to study the effects of polyunsaturated fatty acids on steroidogenesis *in vitro*. For instance, it has been reported that arachidonic acid and eicosapentaenoic acid stimulate testicular testosterone production (Wade and Van Der Kraak, 1993; Wade et al., 1994). As in the case of females, an increase in photoperiod stimulates goldfish testis maturation and this effect seems to be related to the seasonal stage (De Vlaming and Vodienik, 1978). Goldfish testis were also used to study the role of thyroid hormones on reproduction, obtaining similar results to those described for the ovary (Nelson et al., 2010). The observations made from the

mentioned study suggest that T3 acts to diminish steroidogenesis, decrease estrogen synthesis from androgens and decrease sensitivity to estrogen in the gonads. Finally, as mentioned above, goldfish testis has been used as a target tissue to test the role of some water contaminants as endocrine disruptors (Mennigen et al., 2010).

3.9. Heart

One notable feature of the goldfish (and fish in general) heart is that it exhibits an impressive morpho-functional flexibility in response to developmental stages and ecological conditions (Cerra, 2004; Tota and Gattuso, 1996). This cardiac morphology depends on structural arrangements of the ventricular pump and the mechanical function of the heart, and is proportional to the pressure and volume work to the stroke work (Tota and Gattuso, 1996). Based on this feature, goldfish has been studied for cardiac regeneration and remodelling (Grivas et al., 2014). Furthermore, this fish species has been shown to have the capacity to tolerate/sustain severe hypoxic conditions and remain active even during winter in ponds (Bickler and Buck, 2007), actions that require the molecular machinery which sustains myocardial contractility to preserve its function. In order to sustain the condition of hypoxia, lactate is converted to ethanol and carbon-dioxide thereby preventing lactic acidosis and hypoxic/anoxic conditions in goldfish (Bickler and Buck, 2007). Studies related to survival and heart function preservation regardless of oxygen availability has been widely performed using goldfish as a model (Garofalo et al., 2012; Pedersen et al., 2010). Goldfish has also served as a model organism in understanding the hormonal regulation of cardiac functions. For instance, nesfatin-1 has been demonstrated to be a positive inotrope towards the control of cardiac activity in this teleost species (Mazza et al., 2015).

3.10. Gills

The fish gill is an important site for respiratory gas transfer and is dependent on metabolic demand in the environment. It also participates in the uptake of oxygen and release of carbon-dioxide from the body during respiration (Perry et al., 2012). Fish gill has the ability to sustain increased rates of metabolism by regulating larger amounts of gas transfer (Evans et al., 2005). In addition to that, gills contribute to the regulation of other physiological functions, such as ionic and osmotic regulation (Randall et al., 1972). In the case of freshwater fishes, such as goldfish, the maintenance of body fluids is dependent on the environmental conditions and is directly related to the regulation of continuous entry of water and loss of ions facilitated by water to blood diffusion barrier across the gill (Evans et al., 2005). The stimulation of gas transfer and lowering of ion loss is known as osmorepiratory compromise, and can be lowered by dynamically altering the branchial surface area (Randall and Daxboeck, 1984; Sollid et al., 2003). In goldfish, chronic adjustments of functional surface area can be achieved by the presence of an intralamellar cell mass between two lamellae thereby minimizing the impact of osmorepiratory compromise leading to gill remodelling (Sollid et al., 2005). Goldfish has also been considered as a major model organism for understanding the relationship between branchial gas transfer and lamellar surface area in fish gills under acute hypoxia conditions (Nilsson, 2007; Sollid et al., 2003). In a study by Liew and co-workers (2013), the modulation of feeding and swimming on ionoregulation in goldfish was studied by measuring the activity of the gill Na^+/K^+ ATPase. The authors reported that goldfish is able to adapt their osmorepiratory strategy to minimise ions losses whilst maintaining gas exchange under exhaustive swimming. The ion regulatory responses of the goldfish gill has also been studied following metal exposure (Eyckmans et al., 2010). Finally, goldfish has also been studied as a model organism in

understanding the susceptibility of goldfish to *Dactylogyrus intermedius*, an oviparous parasite abundantly found in cyprinid fishes, and the mechanisms involving the protection of fish against adverse parasitic conditions (Lu et al., 2013).

3.11. Integument

The body colour development in goldfish is entirely dependent on the differentiation of chromatophores. The major chromatophores that decide the body colour in goldfish are the black melanophores that consist of melanin, yellow coloured xanthophores containing the pigment pteridine, red erythrophores that contain carotenoids, and guanophores/iridophores containing guanine pigment (Kawamura et al., 1998; Leclercq et al., 2009; Nilsson Sköld et al., 2013; Xu et al., 2005). Among the wide variety of goldfish in terms of skin color, a transparent crucian carp (eg. *C. auratus*) have been identified: a type of goldfish possessing distinct characteristics towards body colour development such as loss of chromatophores pigment from the skin and scales during the depigmentation process in early stages of development leading to transparency of scales, gills and the skin (Xu, 2009). Skin pigmentation is greatly regulated by melanocortin peptides adrenocorticotropin hormone (ACTH) and melanocyte-stimulating hormones (α , β , γ - MSHs), all derived from common family proopiomelanocortin (POMC). Previous reports showed the pigment dispersing activity of α -MSH and its related peptides. Melanocortin receptor subtypes are abundantly expressed in goldfish chromatophores, and mediate classical MSH actions on physiological and morphological color pigmentation in goldfish (Kobayashi et al., 2012). Some studies on the effects of the melanocortin system on color adaptation have been performed in goldfish. For instance, it has been demonstrated that α -MSH stimulates the dispersion of pigments in isolated xanthophores (Kobayashi et al., 2011).

4. Goldfish as a model organism for the study of behavioural responses

Apart from being an ideal model organism in the field of endocrinology, goldfish is one of the fishes most employed for behavioural studies. The use of goldfish for studying behavioural responses started several decades ago. For example, in 1963, Rodgers and Melzack described that pressure wave and visual stimuli can elicit the tail flip response on this fish species. In the same year, Geller reported that hungry goldfish can learn to press a lever for worms, an action that was suppressed in the presence of a flashing light by pairing the light with a brief electric shock and punishing the lever-pressing behaviour with electric shocks.

One of the behavioural aspects most commonly studied using goldfish is locomotor activity. By attaching infrared photocells on the aquaria walls or by using a fixed handycam, many researchers have recorded and quantified the goldfish locomotor activity and studied its possible regulation by external and endocrine factors (Azpeleta et al., 2010; Kang et al., 2011; Matsuda, 2013; Nakamachi et al., 2014; Nisembaum et al., 2014; Vivas et al., 2011; Yahashi et al., 2012). Swimming performance, including spontaneous swimming activity, fast-start swimming performance (a form of burst swimming used by fish during predator-prey encounters) and critical swimming speed (the water speed at which a fish can no longer maintain its position or its maximum prolonged swimming speed, U_{crit}), has also been largely studied in goldfish under, for example, conditions of fin loss (Fu et al., 2013), food deprivation (Liew et al., 2012; Pang et al., 2011), water temperature (Pang et al., 2011), hypoxia (Fu et al., 2011), and exposure to toxicants (Xia et al., 2013).

Together with locomotor activity, feeding is another behaviour that has been extensively studied in goldfish, especially in terms of the enhancing/inhibitory effects that different endocrine signals may exert on it (Abbott and Volkoff, 2011; Bernier and

Peter, 2001; de Pedro et al., 2006; De Pedro et al., 1998; López-Olmeda et al., 2006; López-Patiño et al., 1999; Nakamachi et al., 2006; Unniappan et al., 2004; Volkoff et al., 1999). Other examples of behavioural aspects studied in goldfish include sexual behaviour and chasing (Kawaguchi et al., 2014; Lord et al., 2009), social approach behaviour (Thompson and Walton, 2004), shoaling (Shinozuka and Watanabe, 2004; Xie et al., 2015), and the sediment reworking behaviour (Gandar et al., 2016). In recent years, many characteristics of the goldfish have turned it into a potential non-mammalian model for the study of behavioural neuroscience (Maximino et al., 2015). For example, the scototaxis (dark/light preference) protocol has been validated in goldfish to assess the anti-anxiety effects of pharmacological agents and the behavioural effects of toxic substances, and to investigate the bases of anxiety-related behaviour (Matsuda et al., 2011; Maximino et al., 2010; Nakamachi et al., 2014). Classical fear conditioning methods have also been applied to studies in goldfish (Yoshida et al., 2004). The use of goldfish, and other fish species, as model organisms for the analysis of genetic and biological mechanisms of behavioural neuroscience could offer important comparative information that may eventually be used for the treatment of human psychiatric disorders associated with anxiety, stress and phobic states.

5. Current Trends

Numerous studies have been performed using goldfish as a model organism. Novel methods such as brain mapping studies, *in-situ* hybridization, microarray, gene knockdown (siRNA), gene knockout, tissue-perfusion, developmental studies, and seasonal changes have been performed using goldfish in diverse fields. Also, in the field of endocrinology, goldfish has been used to study the regulation of the growth hormones and regulation of reproductive hormone functions (Unniappan and Peter, 2004). In teleosts, regulation of LH and GH has been well characterized and shown to modulate hormone release in response to intracellular signalling (Canosa et al., 2007; Chang et al., 2012). GnRH has been shown to be widely expressed in goldfish pituitary, and the GnRH receptors play an important role in regulation of neuroendocrine signalling and LH and GH secretion through the action on gonadotrophs and somatotrophs in fish and in other mammalian systems (Grey et al., 2010; Grey and Chang, 2009). By pituitary cell perfusion experiments, it has been shown that growth hormone-releasing hormone (GHRH) is responsible for the stimulation of GH secretion from the goldfish pituitary and acts as a negative neuroendocrine modulator on stimulated LH secretion in goldfish (Grey and Chang, 2013). Also *in situ* hybridization studies using goldfish showed that gonadotropin-inhibitory hormone play an important role in sexual dimorphism of steroidogenesis (Qi et al., 2013). With respect to gene knockdown studies, protein serine/threonine phosphatase (PP-1) gene knockdown has shown to have a critical role in eye formation during goldfish developmental stages (Liu et al., 2012). Brain mapping of ghrelin and ghrelin receptor (*ghs-r1a*) in the goldfish brain and gut showing the interconnection between the ghrelinergic and circadian systems have been performed in goldfish (Sánchez-Bretaña et al., 2015b). Seasonal changes in GH hormone release and its function have been widely studied using

goldfish as model species, and it was found that GH mRNA is elevated during early October but application of goldfish GnIH *in vitro* suppressed basal GH mRNA levels during mid- and later October. Also, gonadotropin-inhibitory hormone (GnIH) suppressed the levels of serum GH release during early recrudescence but not during mid and late recrudescence. These studies suggest goldfish can be considered as an ideal model for endocrine research, but the expression of some hormones is regulated in a seasonal reproductive manner (Moussavi et al., 2014).

6. Challenges

While using goldfish as a model organism in research has numerous advantages, there are also challenges associated with it. Among them, probably the most difficult aspect for genetic studies or for assessing bioactive peptides, is its tetraploid genome (Larhammar and Risinger, 1994). The tetraploidization results in the existence of multiple, additional forms of the same gene which most likely results in more number of protein isoforms. The presence of extra endogenous forms of peptides and its receptors adds further complexity to the already redundant multifactorial endocrine milieu. The existence of a duplicated genome in contrast to mammals and other tetrapod vertebrates with only one genome copy is a common feature in all teleosts, which underwent a whole genome duplication event at the base of their radiation (Meyer and Schartl, 1999; Sidow, 1996). However, goldfish and other cyprinids underwent an additional duplication, thus carrying four copies of their genome (Larhammar and Risinger, 1994). 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, adding additional complexity to the study of genes in goldfish. Goldfish also offers some challenges for studies on reproduction and embryonic development. They reach initial

maturity after 225-233 days (Ortega-Salas and Reyes-Bustamante, 2006), a period considerably longer in comparison to other fishes such as zebrafish and medaka, which reach sexual maturity in 2-4 months (Lawrence et al., 2012). Besides, although larger, goldfish fertilized eggs are softer, thicker and not transparent compared to zebrafish, which make it difficult to carry out developmental studies. Finally, the seasonal and sexual stage must be considered as a potential challenge for some types of endocrine studies, as they have been shown to produce specific variations in the goldfish in terms of the endocrine milieu. For instance, the effects of GnIH on gonadotrope function in the pituitary as well as the regulation of vitellogenin by growth hormone in the liver were reported to be reproductive stage/season-dependent in this species (Moussavi et al., 2009, 2012). It was shown that the expression of antifreeze protein (AFP) gene in goldfish resulted in cold tolerance (Wang et al., 1995).

Conclusions

This review highlights goldfish as a well-characterized model system for studies in integrative physiology and comparative endocrinology. Its ease of handling, low cost of maintenance, anatomical characteristics and its evolutionary position relative to other vertebrates, all makes goldfish one of the fish species most commonly used in research. This has led to a wide knowledge of different aspects of the goldfish physiology and the validation of a large number and diverse techniques for use in this fish. In the post-genomic era, goldfish will continue to shed light on more interesting endocrinology facts. While goldfish is a well-characterized model in endocrinology and neuroendocrinology, the main question that remains is whether we can apply such knowledge directly to other teleosts, or even in mammals. It is possible to apply the knowledge base for some aspects in goldfish on other species, but for some others, it is

likely not applicable. This is largely due to the highly species-specific roles of hormones in fishes. Fundamental research using goldfish, and its outcomes, provide a framework to study endocrine factors in other fishes and/or animals in general. Goldfish is a cultured species. For example, some strains or subspecies were used as ornamental fish, while others are used for food (eg. gibelio). Additional research and new strategies using knowledge generated from endocrine research will also help enhance the commercial importance of this species.

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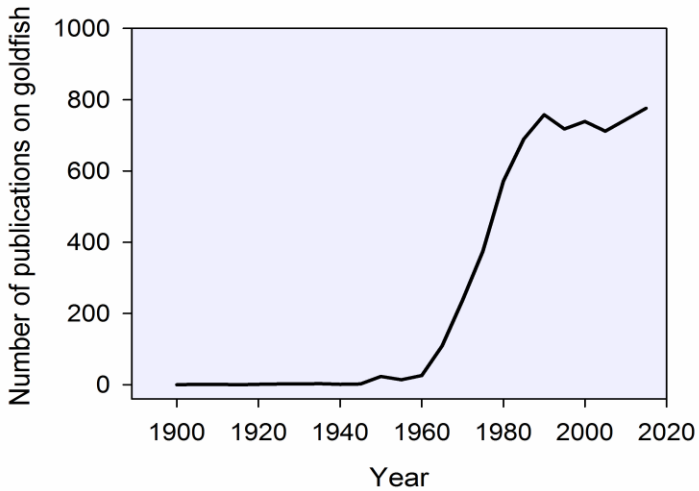
Figure Legends

Figure 1A. Timeline and number of publications using goldfish. The black line represents the number of publications per year on goldfish between 1900 and 2015. This was generated based on a PubMed search of publications containing goldfish in the title or the abstract. **Figure 1B** - asterisks denote the countries from where research using goldfish was reported.

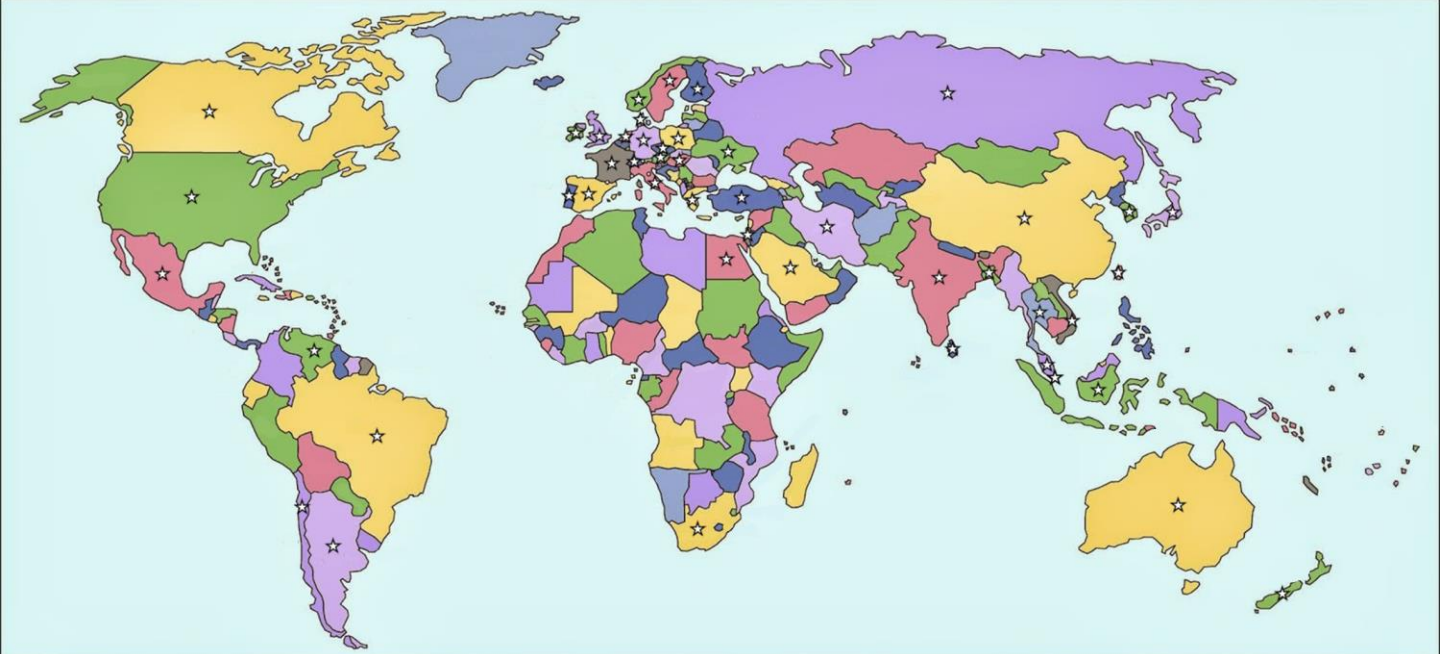
Figure 2A. An artist's rendering of the external anatomy of goldfish. **Figure 2B** shows an artist's rendering of the internal organs in goldfish. Both images were originally drawn for the use of this manuscript. Artist: Juliane Deubner, Saskatoon, Canada.

Figure 2C shows photographs of the external and internal anatomy of goldfish. Individual organs and brain regions are labelled. Female and male fish are shown separately to mark ovary and testis.

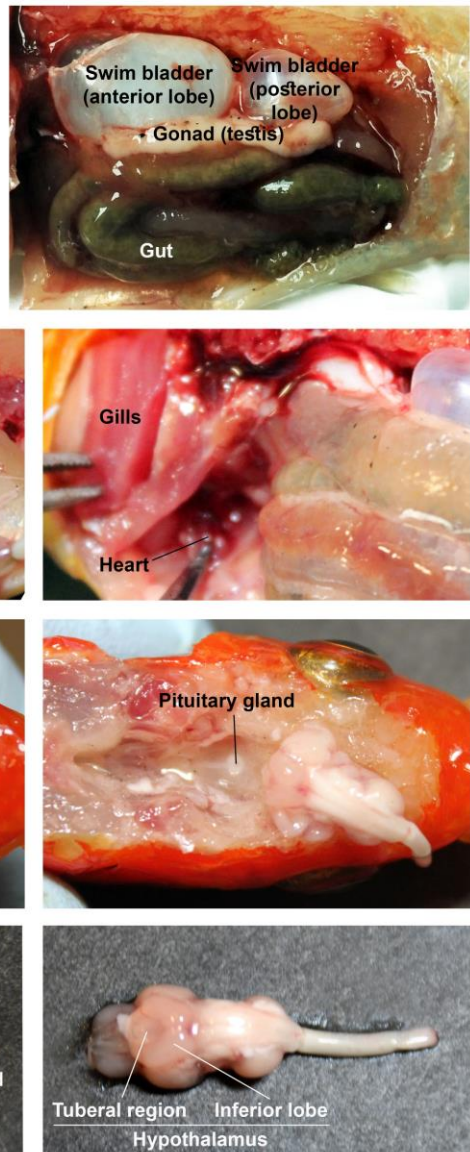
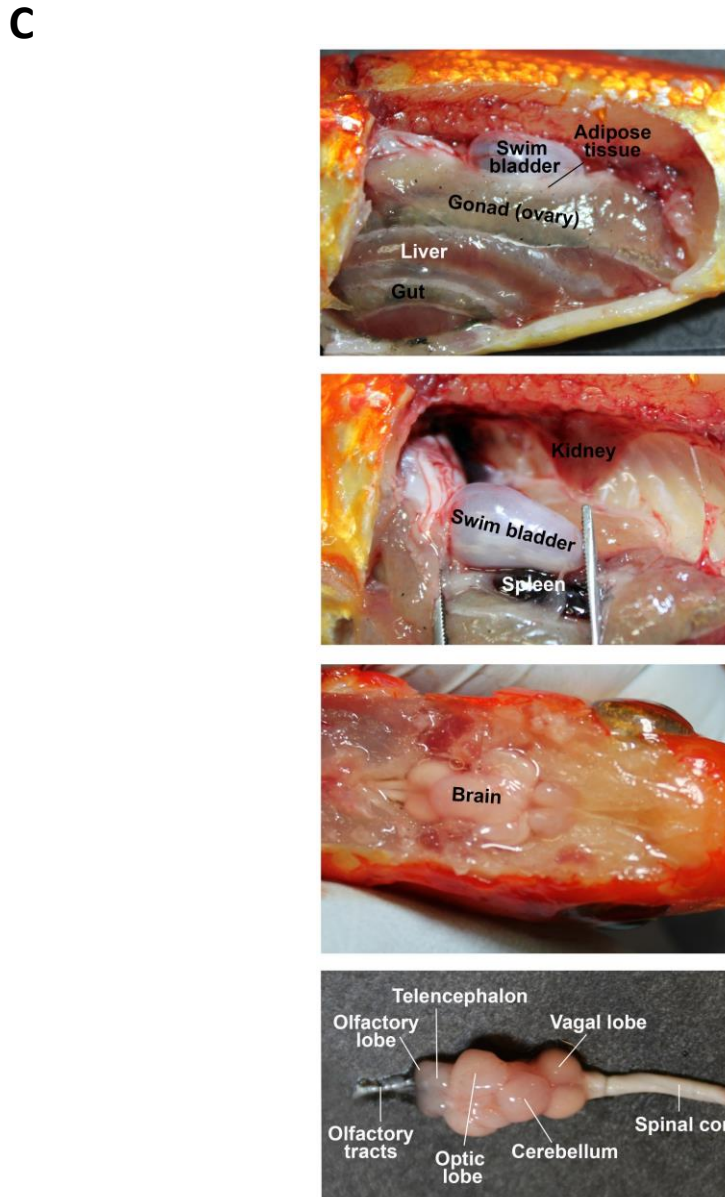
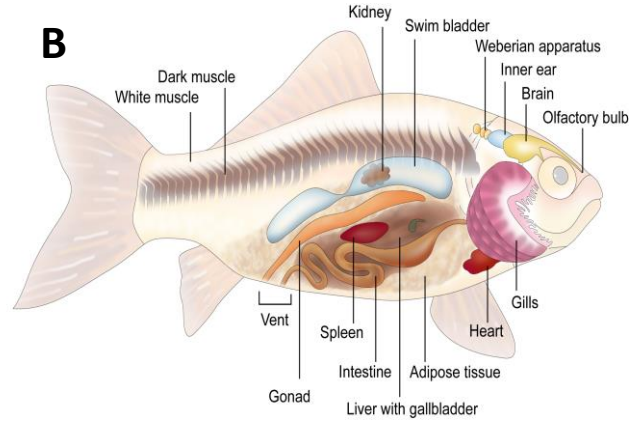
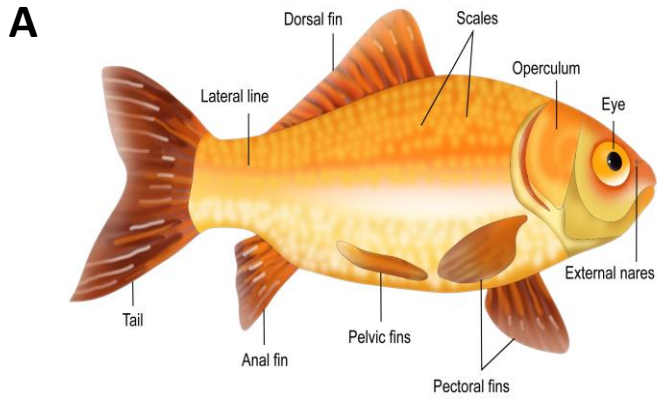
A



B



Blanco et al.,
Figure 2



TABLES

Table 1. Goldfish classification.

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Class	Actinopterygii
Order	Cypriniformes
Family	Cyprinidae
Genus	<i>Carassius</i>
Species	<i>Carassius auratus</i>

ACCEPTED MANUSCRIPT