Leptin signalling in teleost fish with emphasis in food intake regulation

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ABSTRACT

Leptin, the product of the obese (ob or Lep) gene, was first cloned in teleost fish in 2005, more than a decade after its identification in mammals. This was because bony fish and mammalian leptins share a very low amino acid sequence identity, which suggests different functionality of the leptin system in fish compared to that of mammals. Indeed, major differences are evident between the mammalian and fish leptin system. Thus, for instance, mammalian leptin is synthesized and released by the adipose tissue in response to the amount of fat deposits, while several tissues (mainly the liver) are the main sources of leptin in fish, whose determining factors of production are still unclear. In mammals, the main physiological role for leptin is its involvement in the maintenance of energy balance by decreasing food intake and increasing energy expenditure, although a wide variety of actions have been attributed to this hormone (e.g., regulation of lipid and carbohydrate metabolism, reproduction and immune functions). In fish, available literature also points towards a multifunctional nature for leptin, although knowledge on its functions is limited. In this review, we offer an overview of teleostean leptin structure and mechanism of action, and discuss the available knowledge on the role of this hormone in food intake regulation in teleost fish, aiming to provide a comparative overview between the functioning of the teleostean and mammalian leptin systems.

Leptin is a non-glycosylated 16 kDa peptide hormone, product of the obese (ob or Lep) gene, first discovered in mammals in 1994 (Zhang et al., 1994). However, it was not until 2005 when a lep gene orthologue was cloned in a teleostean species (pufferfish, Kurokawa et al., 2005). The reason for this delay lies in a very poor amino acid sequence conservation between bony fish and mammalian leptins (~13–25%). It is one of the peptides showing the lowest fish vs. mammalian sequence identity when compared to other peripheral hormones (e.g., ghrelin: ~45%, Kaiya et al., 2003; PYY: 72%, Söderberg et al., 2002). This low degree of conservation is mainly because bony fish leptins diverged along their own lineage independent of leptins in higher mammals. Instead, the ancestral leptin that gave rise to leptins in mammals and other tetrapods is more closely related to coelacanth and cartilaginous fish leptins (Londraville et al., 2017). The low sequence degree of conservation between bony fish and mammalian leptins points towards a high degree of molecular evolvability and, although the main biological functions for leptin (including food intake regulation) have been described in the different vertebrate groups, this likely suggests the acquisition of distinct biological actions (e.g., role in lipid homeostasis) throughout the course of evolution. This manuscript offers a brief overview of leptin structure and mechanism of action in teleost fish, and reviews the current insights in the involvement of leptin in food intake regulation in this vertebrate group. It also tries to identify current gaps of knowledge to open doors for future investigations in the field. All fish species included in this review, together with the order they belong and their dietary habits, are shown in Table 1. It should be taken into consideration that fish are the most diversified group of vertebrates with 34,300 species identified so far (www.fishbase.org), of which 95% are teleosts, comprising half of all known vertebrate species. In this context, current knowledge on the structure and function of leptin in food intake regulation derives only from some species belonging to 11 of the 40 known teleost orders (Nelson et al., 2006), with no available information for the 29 remaining orders. Considering that teleost fish show a remarkable level of diversity in terms of anatomy, ecology, behaviour, and genome, this lack of knowledge is relevant to make comparisons with other more homogeneous vertebrate groups.

1. Structure

Shortly after a lep gene was identified in pufferfish in 2005

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several fish species from different orders. These include Anguilliformes (European eel, Anguilla anguilla; Japanese eel, Anguilla japonica), Salmoniformes (brown trout, Salmo trutta; rainbow trout, Oncorhynchus mykiss), Characiformes (red-bellied piranha, Pygocentrus nattereri; pacu, Paxu mesopotamicus; red-bellied piranha, Pygocentrus nattereri; Pacu, Paxu mesopotamicus; Red-bellied piranha, Pygocentrus nattereri), Characiformes (zebrafish, Danio rerio; common carp, Cyprinus carpio; grass carp, Ctenopharyngodon idellus; rohu, Labeo rohita; White-clouds mountain minnow, Tanichthys albonubes; Ya-fish, Schizothorax prernanti; Zebrasfish, Danio rerio), Perciformes (chub mackerel, Scomber japonicas; Gilthead seabream, Sparus aurata; Northern snakehead, Channa argus; Red-spotted grouper, Epinephelus akaara; Large yellow croaker, Larimichthys crocea; Japanese sea bass, Lophius chlamys; Red-mouthed croaker, Genyonemus lineatus; Largemouth bass, Micropterus salmoides; Common snapper, Lutjanus apodus; Red-bellied piranha, Pygocentrus nattereri). Bony fish species included in this review with their dietary habits.

| Table 1 | Teleost fish species included in this review with their dietary habits. |
|---------|---------------------------------------------------------------|
| Order | Species | Dietary habit |
| Anguilliformes | European eel, Anguilla anguilla | Carnivorous |
| | Japanese eel, Anguilla japonica | Carnivorous |
| Beloniformes | Medaka, Oryzias latipes | Omnivorous |
| Characiformes | Bucktooth tetra, Hedion neuropterus | Omnivorous |
| | Common carp, Cyprinus carpio | Omnivorous |
| | Crucian carp, Carassius carassius | Omnivorous |
| | Goldfish, Carassius auratus | Omnivorous |
| | Grass carp, Ctenopharyngodon idellus | Herbivorous |
| | Rohu, Labeo rohita | Omnivorous |
| | White-clouds mountain minnow, Tanichthys albonubes | Omnivorous |
| | Ya-fish, Schizothorax prernanti | Omnivorous |
| | Zebrasfish, Danio rerio | Omnivorous |
| Gadiformes | Burbot, Lota lota | Carnivorous |
| Perciformes | Chub mackerel, Scomber japonicas | Omnivorous |
| | Gilthead seabream, Sparus aurata | Carnivorous |
| | Green sunfish, Lepomis cyanellus | Carnivorous |
| | Japanese sea bass, Lutjanus japonicas | Carnivorous |
| | Large yellow croaker, Larimichthys crocea | Carnivorous |
| | Mandarin fish or Chinese perch, Simpeora chuasti | Carnivorous |
| | Mozambique tilapia, Oreoichromis mossambicus | Omnivorous |
| | Murray cod, Macrurus rostratus | Carnivorous |
| | Nile tilapia, Oreoichromis niloticus | Omnivorous |
| | Northern snakehead, Channa argus | Carnivorous |
| | Orange-spotted grouper, Epinephelus coioides | Carnivorous |
| | Red tilapia, Oreoichromis mossambicus x | Omnivorous |
| | Oreochromis aureus | Carnivorous |
| | Red-spotted grouper, Epinephelus acaira | Carnivorous |
| | Striped bass, Morone saxatilis | Carnivorous |
| Pleuronectiformes | Fine Bucker, Paralichthys adspersus | Carnivorous |
| | Tongue sole, Cynoglossus semilaevis | Carnivorous |
| Salmoniformes | Arctic char, Salvelinus alpinus | Carnivorous |
| | Atlantic salmon, Salmo salar | Carnivorous |
| | Brown trout, Salmo trutta | Carnivorous |
| | Chum salmon, Oncorhynchus keta | Carnivorous |
| | Coho salmon, Oncorhynchus kisutch | Carnivorous |
| | Rainbow trout, Oncorhynchus mykiss | Carnivorous |
| Siluriformes | Channel catfish, Ictalurus punctatus | Carnivorous |
| | Yellow catfish, Ptolemaus fuscus | Herbivorous |
| Syngnathiformes | Lined seahorse, Hippocampus erectus | Carnivorous |
| Tetraodontiformes | Pufferfish, Takifugu rubripes | Carnivorous |

(Kurokawa et al., 2005), orthologous gene sequences were described in several fish species from different orders. These include Anguilliformes (European and Japanese eel, respectively, Morin et al., 2015), Cypriniformes (zebrafish, Gorissen et al., 2009; common carp, Huising et al., 2006; grass carp, Li et al., 2010; white-clouds mountain minnow, Chen et al., 2016; Ya-fish, Yuan et al., 2014), Characiformes (red-bellied piranha, Volkoff, 2015; pirapitinga, Volkoff, 2015; pacu, Volkoff et al., 2017; cavefish, Jeffery, 2020), Siluriformes (yellow catfish, Gong, 2013), Salmoniformes (brown trout, Angotzi et al., 2013; rainbow trout, Murashita et al., 2008; Atlantic salmon, Angotzi et al., 2013 and Rannestad et al., 2010; Arctic char, Angotzi, 2013 and Frilland et al., 2010), Pleuronectiformes (tongue sole, Xu et al., 2018), Beloniformes (medaka, Kurokawa and Murashita, 2009), Perciformes (Nile tilapia, Shpilman et al., 2014; striped bass, Won et al., 2012; orange-spotted grouper, Zhang et al., 2013; chub mackerel, Ohga et al., 2015; mandarin fish or Chinese perch, He et al., 2013 and Yuan et al., 2016; Northern snakehead, Wen et al., 2020), and Syngnathiformes (lined seahorse, Zhang et al., 2016).

Most bony fish species (e.g., tongue sole, Nile tilapia, orange-spotted grouper, chub mackerel, Northern snakehead, zebrafish, medaka) have two, sometimes highly divergent, lep-a and lep-b (Chen et al., 2016; Gorissen et al., 2009; Kurokawa and Murashita, 2009; Ohga et al., 2015; Shpilman et al., 2014; Wen et al., 2020; Xu et al., 2018; Yuan et al., 2016; Zhang et al., 2013). This is the result of the whole-genome duplication (WGD) event, which occurred early in the teleost lineage (3R) (Glasauer and Neuhaus, 2014). A schematic representation of vertebrate evolution and number of lep paralogues identified in the main groups is shown in Fig. 1. The amino acid identity is low between Lep a and Lep b (20–30%) and phylogenetic analysis shows that the two genes can be classified into separate clades (Deck et al., 2017). Of both leptins, the A form is the predominantly expressed in most of the fish species examined so far, showing 10–100 times greater tissue mRNA copy number than lep-b and hence likely reflecting the major source of circulating leptin (Deck et al., 2017). Furthermore, the additional WGD or tetraploidisation (4R) in some teleost groups, such as carps and salmonids (Glasauer and Neuhaus, 2014) resulted in up to four lep paralogues in these species (Angotzi et al., 2013). Since lep-a sequences and lep-b sequences in these species are reasonably comparable (71–83%) they are therefore referred to as lep-a/L and lep-b/L. On the contrary, other fish groups or species (e.g., mandarin fish, pufferfish) underwent genome reduction and retained only one lep gene (He et al., 2013; Kurokawa et al., 2005). This context of differential rates of gene loss or divergence among lineages, together with the limited availability of sequence data, makes challenging to decipher and understand the phylogeny of the lep gene in teleost fish. While comprehensive and very interesting reviews on its phylogenetic evolution are available in the literature (Denver et al., 2011; Gorissen and Flik, 2014; Huising et al., 2006b), several gaps of knowledge regarding the function of different isoforms still remain and warrant further investigation.

Bony fish lep shares a very low sequence identity (~13–25%) with the mammalian Lep. However, despite the high divergence in primary structure, fish and other vertebrate leptins show a conserved gene structure. They are highly similar in their predicted secondary and tertiary structures (when modelled based on the crystal structure of human leptin) and they conserve key amino acids required for biological activity (Huising et al., 2006a; Kurokawa et al., 2005; Rannestad et al., 2010), demonstrating an evident orthology. In this sense, fish leptins are encoded by two exons that in some species (e.g., common carp) have a very similar length to the corresponding exons of the human and mouse Lep (Huising et al., 2006a). The secondary structure of fish leptin, like mammalian leptin, is predicted to comprise four antiparallel helices (named A to D) with a small helical segment (helix E) in the loop linking helices C and D as shown in Fig. 2. Finally, fish leptins contain a conserved pair of cysteine residues that in the mammalian leptin forms a disulphide bridge connecting the C-terminal ends of helix C and D (Denver et al., 2011; Wen et al., 2020). A third cysteine has been predicted in zebrafish lep-b (Gorissen et al., 2009), but this residue has not been found in medaka (Kurokawa and Murashita, 2009) and Atlantic salmon (Rannestad et al., 2010), suggesting it to be species-specific. This cysteine has been proposed to be responsible for some of the differentiation observed between the functions of the two leptins in zebrafish (Gorissen et al., 2009). While fish leptins conserve the pair of cysteine residues forming the disulphide bridge, it is of note that they lack the six residue motif GLDFIP (positions 38 to 43 in human leptin) completely conserved among tetrapods and required for activation of the leptin receptor (Denver et al., 2011). The absence of this motif in fish may indicate a different mechanism for binding to and activation of the leptin receptor in these vertebrates compared with tetrapods.
Contribute to leptin production, as demonstrated in rainbow trout adipocytes (Salmeron et al., 2015). In species with two leptin forms, the major leptin hepatic expression is in some cases displayed by only one leptin form, while the other shows a differential pattern of expression. For instance, in common carp, the liver expresses the highest mRNA levels of lept-b but very low levels of lept-a; instead, lept-a is predominantly expressed in thymus, kidney, and spleen (Huising et al., 2006a). Conversely, in northern snakehead, the liver shows the highest expression levels of lept-a but almost undetectable expression of lept-b mRNAs, which are almost restrictedly expressed in the brain (Wen et al., 2020). Likewise, lept-a is more abundant in the liver of adult medaka, but lept-b shows its highest expression levels in the brain and eye (Kurokawa and Murashita, 2009). In mandarin fish, expression of lept-a is almost restricted to the liver; however, lept-b hepatic expression is very low, and is instead more predominant in the gill and adipose tissue (Yuan et al., 2016). Finally, the liver expresses important levels of zebrafish lept-a mRNAs, but not of lept-b mRNAs, which are predominantly expressed in the ovary. It should be noted that while the liver is an important site for lept-a expression in zebrafish, similar amounts of mRNAs have been detected in the pituitary gland, gills, heart, spleen, gut and ovary, suggesting other tissues to also be main sources for lept-a production in this species (Gorissen et al., 2009). Similarly, the liver and gonads are both important sites for lept-al expression in the goldfish (Carassius auratus), followed by the brain pituitary, spleen, foregut, and kidney (Tinoco et al., 2012). Additional major sites for lept expression have been also...
Fig. 3. Proposed mechanism of action underlying the anorectic role of leptin in fish. Under situations of high fuel stores (glucose? lipids?) or low plasma leptin levels, the liver (and/or other leptin sources depending on the species) synthesizes and releases leptin, which enters into circulation to reach the hypothalamus, where it binds to its receptor (LeptR) located in the cell membranes. The binding of leptin to LepR activates the Jak/STAT intracellular signalling pathway, which leads to the downregulation of the expression of the orexigens NPY and AGRP and the upregulation of the anorexigens POMC and CART in the nucleus. Such changes in hypothalamic neuropeptides ultimately result in a decrease in food intake levels. Agp, agouti-related protein; Cart, cocaine- and amphetamine-regulated transcript; Lep, leptin; LepR, leptin receptor; Npy, neuropeptide Y; Pomc, proopiomelanocortin.

reported in other fish species, e.g., the skin in Nile tilapia (Liu et al., 2018). Indeed, in few species, the liver is not the predominant leptin-expressing location and other tissues different to the liver appear to be major sources for this hormone. This is the case, for instance, of the European eel, in which the pituitary and eye express the highest levels of lep-a and lep-b, respectively (Morini et al., 2015), and the red-bellied piranha, in which the major sites for expression are the heart, spleen, pituitary and kidney (Helene Volkoff, 2015). Overall, while the liver is typically considered the major producer of leptin in fish, it is clear that important species-specific differences in leptin sources exist among species and therefore caution should be taken when comparing available literature or selecting target tissues for further research.

The synthesis of leptin in fish responds to a variety of factors. Among them, fasting is an important modulator of leptin circulating levels and expression. Nevertheless, controversial and opposite observations have been reported depending on the species, resulting in three different response patterns. In a first pattern, food deprivation leads to reduced levels of leptin in plasma, as observed in fasted green sunfish (Johnson et al., 2000) and burbot (Nieminen et al., 2003), consistent with the fasting-induced decrease in circulating leptin in mammals (Ahima et al., 1996). This is also in agreement with the decreased levels of lep-b mRNA expression reported in the liver of zebrafish after 1 week of fasting (Gorissen et al., 2009), and of lep mRNA abundance in the liver of fasted ya-fish (Yuan et al., 2014), food-deprived striped bass (Won et al., 2012) and food-restricted grass carp (Gong et al., 2017), and in the intestine of fasted red-bellied piranha (Volkoff, 2015), zebrafish (Garcia-Suarez et al., 2018), and bucktooth tetra (Butt et al., 2019). Opposite to these observations, a second pattern of response characterized by a rise in plasma leptin levels upon different periods of fasting. This occurred in rainbow trout (Gong et al., 2013; Kling et al., 2009), Atlantic salmon (Trombley et al., 2012), Arctic char (Jorgensen et al., 2013), Mozambique tilapia (Douros et al., 2017), chub mackerel (Ohga et al., 2015), and fine flounder (Fuentes et al., 2012). Opposite to these observations, a second pattern of response characterized by a rise in plasma leptin levels upon different periods of fasting. This occurred in rainbow trout (Gong et al., 2013; Kling et al., 2009), Atlantic salmon (Trombley et al., 2012), Arctic char (Jorgensen et al., 2013), Mozambique tilapia (Douros et al., 2017), chub mackerel (Ohga et al., 2015), and fine flounder (Fuentes et al., 2012). This response goes in line with the upregulated liver expression of lep-a in 24-h fasted mandarin fish (Yuan et al., 2016), 1-week food-deprived lined seahorse (Zhang et al., 2016), 2-week food-deprived Northern snakehead (Wen et al., 2020), 20-day fasted Mozambique tilapia (Douros et al., 2017), and of lep-a and lep-b in white-clouds mountain minnow in response to short- and long-term fasting (Chen et al., 2016), and of lep-a1 and lep-a2 in Arctic char in response to long-term fasting (Jorgensen et al., 2013). Also under conditions of food restriction, a rise in plasma leptin levels in rainbow trout at 8 weeks (Salermon et al., 2015) and increased lep mRNA abundance in the Atlantic salmon liver (Moen and Finn, 2013; Trombley et al., 2014) were reported. Finally, in a third pattern of response, food deprivation does not modulate leptin synthesis and/or expression. Thus, in zebrafish (Gorissen et al., 2009) and goldfish (Tinoco et al., 2012), no difference in hepatic lep-a and lep-al (respectively) expression was observed between 1-week fasted and fed fish. Likewise, hepatic lep-a and lep-b expression was observed to not respond to a short- (7 days) or long-term (6 weeks) fasting period or to subsequent refeeding in common carp (Huising et al., 2006a). A similar lack of fasting-induced modulation of the fish leptin system occurred under very long-term periods of food deprivation. Thus, fasting during 4 months did not alter plasma leptin levels in rainbow trout (Jorgensen et al., 2016) or lep-a and lep-b expression in the liver of European and Japanese eel (Morini et al., 2015), suggesting that the actions of the hormone operate at short and not long-term. With these varied response patterns, it is very difficult to make a generalization about leptin behaviour in response to food deprivation, but certainly a clear difference exists when comparing teleosts with the single pattern known in mammals.

Feeding should induce in leptin synthesis changes opposed to those elicited by food deprivation. Considering that fasting alters leptin expression or circulating levels in a different way depending on the species (see above), it is not surprising to see comparable differences in the response of leptin to feeding. Thus, feeding/re-feeding down-regulates circulating levels of leptin in rainbow trout (Johansson and Brommson, 2015) and fine flounder (Fuentes et al., 2012), and lep mRNA abundance in the liver of orange-spotted grouper (Zhang et al., 2013) and chub mackerel (Ohga et al., 2015). In contrast, hepatic lep expression increased with feeding in goldfish (Tinoco et al., 2012), common carp (Huising et al., 2006a), striped bass (Won et al., 2012) and mandarin fish (Yuan et al., 2016). Finally, lep expression did not change in response to feeding in grass carp (Liang et al., 2019b). It is not clear why divergent responses occur but this is again suggestive of a clear different behaviour compared with mammals.
In addition to fasting and feeding, altered leptin expression/secretion occurred in fish in response to diet composition. For instance, in grass carp, a diet rich in carbohydrates (Cai et al., 2018) or fats (Li et al., 2016) increased leptin mRNA abundance in the liver. Likewise, Nile tilapia fed a high-carbohydrate or a high-fat diet showed elevated plasma leptin levels, accompanied by increased expression of lep-a mRNAs and Lep-A protein in subcutaneous adipose tissue and liver, respectively (Liu et al., 2018). In blunt snout bream, increased levels of leptin in plasma occurred in parallel with decreased food intake when fish were fed a high-fat diet (Dai et al., 2018). Large yellow croaker fed a diet rich in palm oil have higher leptin levels in serum than fish fed a diet rich in fish oil (Wang et al., 2019). Similarly, the dietary shift from fish oil to vegetable oil resulted in higher levels of leptin in plasma in rainbow trout (Francis et al., 2014), while reduced leptin content was reported in grass carp fed a diet based on plant protein blend compared to a diet based on fish meal (Li et al., 2019a). On the contrary, no differences in plasma leptin levels were detected in gilthead sea bream (Ganga et al., 2005), rainbow trout (Ettore et al., 2012) and Murray cod (Ettore et al., 2012) in response to the substitution (complete or partial) of fish oil by vegetable oil in the diet. A similar lack of changes in response to diet composition was observed for the hepatic expression of leptin in red tilapia fed diets with different protein content (Santos et al., 2020), in GIFT tilapia fed high plant-based diets (Zou et al., 2017), and in gilthead sea bream fed a high-carbohydrate, high-protein or high-fat diet (Babaei et al., 2017). Likewise, lep mRNA levels remained unaltered in the intestine of pacu when fed diets with a different soy protein concentration (Volkoff et al., 2017), and in the brain and gut of zebrafish fed a diet with high levels of plant protein (Kwasek et al., 2020). With this background, additional studies are required on the effects of different diet composition on leptin gene and protein levels to better understand the meaning of circulating leptin under different conditions of energy status.

Evironmental factors such as hypoxia, water temperature, and water salinity are important modulators of leptin expression in fish. Thus, exposure to hypoxic conditions increased leptin-a mRNA levels in the liver of common carp (Bernier et al., 2012) and zebrafish (Chu et al., 2010), and in zebrafish embryos (Yu et al., 2012), as it does in mammals (Bruder et al., 2005; Wang et al., 2008). A similar pattern of up-regulation of leptin-a mRNAs was present in zebrafish embryos exposed to cobalt chloride (Chu et al., 2016; Yu et al., 2012), a chemical inducer of hypoxia-inducible factor 1 (HIF-1), a transcriptional factor that has been reported to mediate the transcriptional response of leptin to hypoxia (Chu et al., 2010). Likewise, increased plasma leptin levels and hepatic leptin-a mRNA abundance were detected in rainbow trout in response to induced hypoxemia (MacDonald et al., 2014). As for temperature, a significant increase in leptin-a and leptin-b mRNA levels in liver occurred in goldfish when water temperature decreased from 28 °C to 15 °C (Chen et al., 2019). The rise in temperature in red-spotted grouper from 15 °C to 20 °C resulted in increased leptin-a mRNA levels in the liver and decreased levels in the brain (this latter effect was also observed in fish reared at 25 °C), with no significant changes observed for leptin-b. These changes in expression were accompanied by increased (not decreased as expected) food intake levels (Jeon et al., 2020). Finally, no differences in leptin levels in plasma or liver were detected in burbot kept at 2 °C or 10 °C (Nieminen et al., 2003). Acclimation to different salinities also altered leptin levels, as observed in Mozambique tilapia, in which an increase in hepatic leptin-a mRNAs occurred when fish were transferred from freshwater to seawater (Saltzgar et al., 2014). Leptin plasma levels and hepatic leptin-a mRNAs also increased in channel catfish following transfer from seawater to freshwater (Choi et al., 2014). In Arctic charr, however, increased salinities did not affect plasma leptin levels (Arnason et al., 2014). Changes in all these environmental parameters may also result in a stressful situation for fish. Considering that stress in fish generally leads to an anorectic response (Conde-Sieira et al., 2018) and that leptin levels and mRNA abundance generally increase under these situations (see above), we may suggest that at least part of the changes in food intake during stress situations might relate to the activation of the leptin system. This is supported by responses observed under stressful situations like hypoxia in rainbow trout (MacDonald et al., 2014) and the inflammatory response elicited in the intestine of blunt snout bream as a result of feeding on a high-fat diet (Dai et al., 2018). Furthermore, Madison and coworkers found that cortisol treatment enhanced leptin-a mRNA abundance in the rainbow trout liver (Madison et al., 2015).

The circadian system modulates leptin synthesis and/or activity as supported by four findings. First, the existence of circadian changes in expression of leptin genes in the liver and/or brain of goldfish (Tinoco et al., 2014) and zebrafish (Paredes et al., 2015), which are highly dependent on photoperiod. Second, mRNA abundance of leptin receptor A1 (lep-rA1) changed under different photoperiods in the hypothalamus of Atlantic salmon (Chi et al., 2019). Third, leptin is only effective in modulating food intake and locomotor activity in goldfish when administered during the photophase (Vivas et al., 2011). Fourth, the dysregulation in the circadian activity of the leptin system observed in lep-a KO zebrafish (Audira et al., 2018). In contrast with the apparently clear circadian activity, there is controversy regarding the existence of a seasonal modulation of the leptin system. Thus, in Arctic charr plasma leptin levels and hepatic lep expression did not change significantly during the annual feeding cycle, despite the marked differences in feeding and body fat detected throughout the cycle (Froiland et al., 2012). However, other studies in the same species revealed important changes in lep mRNA abundance in the brain (Striburny et al., 2015) and liver (Froiland et al., 2010) during a seasonal feeding cycle. In Atlantic salmon, enhanced expression of both lep-a and lep-b in liver occurred in winter, which might relate to the reduced food intake observed in that period (Moen and Finn, 2013).

Reproduction and reproductive hormones also relate to the modulation of leptin synthesis in fish. Thus, estrogen increased leptin-a (but not lept-b) mRNA expression in the liver of male and female white-clouds mountains minnow when administered intraperitoneally or added into the water (Chen et al., 2016). Increased levels of leptin-a (but not lept-all) mRNAs occurred in Atlantic salmon implanted with capsules containing testosterone (Trombley et al., 2018). Likewise, abundance of leptin-aI and leptin-all upregulated in primary cultures of Atlantic salmon hepatocytes upon treatment with estradiol, testosterone and 11-ketotestosterone (Trombley et al., 2015). However, in tongue sole, estradiol or testosterone treatment resulted in decreased mRNA abundance of leptin-a in cultured hepatocytes, with no changes in leptin-b (Xu et al., 2018). These effects of sex steroids support the reproduction-related changes in the leptin system observed in several species, such as the involvement of leptin in sexual dimorphism in yellow catfish (Zhang et al., 2017) or the enhanced expression of leptin-a during maturation in lined seahorse (Zhang et al., 2016).

Finally, synthesis of leptin is subject to an endocrine modulation. One of such leptin-regulating hormones in fish appears to be pituitary growth hormone (GH). Douros and coworkers demonstrated that abolition of pituitary GH via hypophysectomy in Mozambique tilapia increases hepatic leptin-a mRNA abundance, which returns to levels similar to that of sham control after GH replacement (Douros et al., 2017). Accordingly, the same authors reported a decrease in leptin-a expression in response to treatment with GH in Mozambique tilapia primary hepatocytes; however, this effect was dose-dependent. The opposite response was detected with high GH concentrations (Douros et al., 2017). The effect of GH on leptin synthesis is also supported by the finding of decreased lep-a mRNA abundance in adipose tissue (although not in liver) of transgenic cohoh salmon with enhanced GH production (Kim et al., 2018). Besides GH, there is some evidence in favor of a role for other hormones in modulating leptin synthesis. For instance, treatment with ghrelin in rohu (Dar et al., 2020) and with neuropeptide Y (NPY) in grass carp (Zhou et al., 2013) reduced leptin mRNA abundance in the liver. Melatonin administration also decreased lep mRNA in the zebrafish brain (Montalbano et al., 2018). On the contrary, other hormones appear to induce the leptin system upon administration. Thus, insulin
treatment enhanced leptin production in rainbow trout adipocytes (Salmerón et al., 2015) and lep expression in grass carp hepatocytes (Lu et al., 2015). The thyroid hormone T3 also increased lep expression in grass carp hepatocytes (Lu et al., 2015). The treatment with brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 21 (FGF21) increased hepatic lep-a and lep-b mRNA levels in zebrafish (Blanco et al., 2020a, 2020b). Finally, administration of palmitoleylthanolamide resulted in a rise in lep-al mRNA abundance in the liver of goldfish (Gómez-Boronat et al., 2020).

3. Receptors and mechanism of action

The actions of leptin are mediated by its binding to the leptin receptor (LepR), a member of the class I helical cytokine receptor family (Liong and Ward, 2007). In teleost fish, an orthologous gene for the mammalian LepR has been identified in several species. These include zebrafish (Liu et al., 2010), medaka (Kurokawa and Murashita, 2009), goldfish (Tinoco et al., 2012), crucian carp (Cao et al., 2011), rainbow trout (Gong et al., 2013), Atlantic salmon (Rønnestad et al., 2010), Nile tilapia (Shpilman et al., 2014), yellow catfish (Gong et al., 2013), yellow catfish (Gong et al., 2013), and black bream (Zhang et al., 2016), among others. LepR is typically present as a single orthologue; however, duplicate paralogues have been identified in a few species, such as the Atlantic salmon (Angotzi et al., 2016) or the European eel (Morini et al., 2015). Furthermore, in some species more than one isoforms of LepR is present. For instance, in addition to the long, functional form, four shorter LepR isoforms are present in the Atlantic salmon as a result of alternative splicing at the 3′ end of the gene transcript (Rønnestad et al., 2010). This is similar to that described in mammals, in which at least six distinct mRNA transcripts producing a variety of LepR protein isoforms were described (Zabeau et al., 2003). Four shorter receptor isoforms have only intracellular domains and thus are apparently able to bind circulating leptin (Rønnestad et al., 2010). Likewise, Cao and coworkers cloned three leptin receptor isoforms in the crucian carp: the long form (cclp-L), a short form containing identical extracellular and transmembrane domains to cclp-L but diverging in sequence thereafter (cclp-s1), and an isoform containing only extracellular domains (secreted form, cclp-s2) (Cao et al., 2011). Finally, in addition to the long form, a truncated (LepRc) and three shorter (LepR5, LepR6, and LepR3) leptin receptor isoforms were described in rainbow trout (Gong et al., 2013).

Structurally, LepRs typically have an extracellular ligand binding domain and a cytoplasmic tail that mediates intracellular signalling upon leptin binding (Denver et al., 2011, Fig. 3). In mammals, leptin interacts with its receptors through three different binding sites, named I-III (Peelman et al., 2004). These binding sites are well characterized in mammals (Peelman et al., 2004, 2006), but scarce information is available in fish. Rønnestad and coworkers reported that identity between lepR sequences of Atlantic salmon and mammals is very low. However, the salmon LepR (long form) includes all functionally important domains conserved among vertebrate LepRs: three fibronectin type III (FN III) domains, the immunoglobulin (Ig) C2-like domain, a pair of repeated tryptophan/serine motifs (WSXWS) at an extracellular segment, two JAK2-binding motif boxes, and a STAT-binding domain at an intracellular segment (Denver et al., 2011; Rønnestad et al., 2010). In contrast, authors reported that the leptin-binding domain of the Atlantic salmon LepR shares only 31.2% amino acid sequence identity with that of human receptor (Rønnestad et al., 2010). This was later supported by Prokop and coworkers, who also reported high variation in the binding interface of leptin to LepR between fish and mammals (Prokop et al., 2012). Despite such a variation in the leptin-binding sites, authors described that all vertebrate LepR sequences have 32 conserved amino acids (25 functionally conserved), including two disulfide bridges and an N-glycosylated asparagine (amino acid 194), and that binding sites I and II are highly conserved (Deck et al., 2017; Prokop et al., 2012).

LepRs shows, in general, a widespread distribution within fish tissues (Gong et al., 2013; Kurokawa and Murashita, 2009; Morini et al., 2015; Ohga et al., 2015; Rønnestad et al., 2010; Shpilman et al., 2014; Tinoco et al., 2012; Zhang et al., 2013). The highest expression levels are detected in the brain, pituitary and gonads, as described in several species, such as goldfish (Tinoco et al., 2012), Atlantic salmon (Rønnestad et al., 2010), and European eel (Morini et al., 2015). Nevertheless, there are species-specific differences in the tissue distribution of lepR mRNAs. For instance, in Nile tilapia, the pituitary (but not the brain and gonads) expresses high levels of lepR mRNAs; instead, the muscle, head kidney and pancreas are also major expression sites (Shpilman et al., 2014). In medaka, lepR transcripts are more abundant in muscle, followed by the skin gill, brain and eye (Kurokawa and Murashita, 2009). A high expression levels of lepR mRNAs in head kidney, together with the kidney, are also present in the orange-spotted grouper (Zhang et al., 2013). Such divergence in the tissue expression profile of lepR among species points towards the putative existence of different functions for leptin depending on the species.

The expression of lepR in fish changes in response to several factors. One of such factors is the nutritional status. In this regard, an increase in lepR mRNA abundance occurred in liver of rainbow trout in response to 2 weeks of fasting (Gong et al., 2013) and in the brain of Atlantic salmon fed a restricted ration (Angotzi et al., 2016). Conversely, decreased lepR mRNA levels in the brain, accompanied with decreased lepa mRNA levels in the liver, occurred in chub mackerel fed 3 times/day compared to those fed once daily (Ohga et al., 2015). In Japanese sea bass, feeding a plant-enriched diet, a situation inducing anorexia, also decreased lepR mRNA abundance in the hypothalamus (Li et al., 2019b). Besides the nutritional status, some reproductive hormones affect lepR expression. Thus, treatment with oestradiol or testosterone significantly decreased lepR mRNA abundance in tongue sole hepatocytes (Xu et al., 2018). Finally, brain and hepatic lepra1 (but not lepra2) mRNA levels increased during progression of life stages in Atlantic salmon (Angotzi et al., 2016).

LepR, like all class I helical cytokine receptors, signals via the Janus kinase/signal transducers and activators of transcription (Jak/STAT) pathway (O’Sullivan et al., 2007). The Jak (Jak2) and STAT (STAT3 and STAT5) proteins important for LepR signalling are highly conserved across vertebrates (Denver et al., 2011). In mammals, leptin binding to LepR induces the autophosphorylation of Jak2, which in turn phosphorylates three tyrosine residues (Y985, Y1077 and Y1138) located in the cytoplasmic domain of LepR. The phosphorylated Jak2s and tyrosines serve as docking sites for signal-transduction proteins bearing a phosphorylating binding domain called Src homology 2 (SH2). Particularly, phosphorylation of Y985 leads to recruitment of SH2-containing tyrosine phosphatase-2 (SHP2) and subsequent activation of the extracellular signal-regulated kinase (ERK); phosphorylation of Y1077 leads to recruitment and phosphorylation of STAT5; and phosphorylation of Y1138 leads to recruitment and phosphorylation of STAT3 (Villanueva and Myers, 2008). The exact mechanism of action triggered after leptin binding to LepR is mostly unknown in teleost fish. Available studies in rainbow trout (Aguilar et al., 2011; Gong et al., 2016) demonstrated that the presence of specific inhibitors of Jak/STAT and IRS-PI3K pathways reverted the effects of leptin treatment in hypothalamus. This evidence, together with the high degree of homology in key motifs critical for recruitment and activation of Jak/STAT proteins between fish and mammals (de Kok et al., 2017; Shpilman et al., 2014), points towards conserved mechanisms to those described in mammals (Fig. 3). However, since available studies refer only to one fish species it is too early to make any generalization regarding this specific topic.

4. Role of leptin in food intake regulation

Leptin in mammals is involved in the regulation of a wide variety of physiological processes, including food intake, energy expenditure, lipid
and carbohydrate metabolism, reproduction and immune functions, among others (Friedman, 2014, 2019). In teleost fish, the knowledge on leptin functions is more limited. Available literature points towards leptin as a multifunctional hormone in fish (Gorissen and Flik, 2014; van de Pol et al., 2017) as it is in mammals. However, it is not always possible to make generalizations of comparable roles between fish and mammals. One of the most important roles of leptin is its involvement in the maintenance of energy balance by regulating food intake and energy expenditure. For this, in mammals, leptin is synthesized and released by adipose tissue when lipid stores are available, in an amount proportional to the size of fat depots, thus functioning as a lipostat (Considine et al., 1996; Maffei et al., 1995). In fish, what determines leptin production remains scarcely assessed and available studies evaluating leptin responsiveness to metabolic state are equivocal. However, the presence of high fat stores associated with low plasma leptin levels, and high glucose levels appear to be possible determinants of leptin secretion (Fig. 3), as observed in Atlantic salmon (Trombley et al., 2014) and grass carp (Lu et al., 2015), respectively. However, unlike mammals, leptin in fish does not seem to act as an adipostatic factor but available studies suggest that this hormone relates to glucose homeostasis. Thus, Michel and coworkers (Michel et al., 2016) proposed that leptin in fish acts as a glucostat, based on the differences in parameters related to glucose metabolism observed in lepr knockout (KO) zebrafish when compared with wild type (WT). It is interesting to mention that lepr KO zebrafish are not developing obesity (Michel et al., 2016), which is in marked contrast with mammals, particularly mice (ob and db models). This again illustrates the differences between leptin synthesis in fish and mammals on a functional standpoint. The hypothesis of leptin acting as a glucostat in fish is also supported by three indirect findings. First, the effects of glucose on leptin synthesis and expression in grass carp (Lu et al., 2015). Second, the actions of leptin on glucose homeostasis in Nile tilapia (Liu et al., 2018), and on hepatic glucose metabolism of Mozambique tilapia (Baltzegar et al., 2014) and mandarin fish (Yuan et al., 2020). Third, the effects of leptin on the glucosensing capacity of

| Table 2 | Effects of leptin (homologous: hom., or heterologous: het.) treatment, or knockout (KO) of leptin-related genes, on food intake and the hypothalamic mRNA abundance of orexigenic (NPY, AgRP) and anorexigenic (POMC, CART) neuropeptides in different fish species. |
| Species | Treatment | Dose | Source | Time | Food intake | NPY | AgRP | POMCa | CART | Reference |
| Atlantic salmon | Minipump | 0.1–10 ng/g/h | hom: leptin A1 | 20 days | ▲ | ▲ | ▲ | ▲ | ▲ | Murashita et al. (2011) |
| Channel catfish | IVC | 40 ng/g | het: rat | 1h | ▲ | ▲ | ▲ | ▲ | ▲ | Silverstein and Plisetskaya (2000) |
| Coho salmon | Minipump | 14 ng/g/h | het: human | 2 weeks | ▲ | ▲ | ▲ | ▲ | ▲ | Baker et al. (2000) |
| Goldfish | IVC | 1 and 5 ng/g | het: murine | 1h | ▲ | ▲ | ▲ | ▲ | ▲ | Volkoff and Peter (2001) |
| | 10 ng/g | het: murine | 6h | ▲ | ▲ | ▲ | ▲ | ▲ | ▲ | Volkoff and Peter (2001) |
| | 1–100 ng/g | het: murine | 1h | ↓ | ↓ | ↓ | ↓ | ↓ | de Pedro et al. (2006) |
| | 10 ng/g | het: murine | 2 and 6h | ↓ | ↓ | ↓ | ↓ | ↓ | de Pedro et al. (2006) |
| | 100–1000 ng/g | het: human | 2 and 8h | ▲ | ▲ | ▲ | ▲ | ▲ | de Pedro et al. (2006) |
| | IP | 100–400 ng/g | het: murine | 1h | ↓ | ↓ | ↓ | ↓ | ↓ | de Pedro et al. (2006) |
| | IP | 100–3300 ng/g | het: human | 2 and 8h | ▲ | ▲ | ▲ | ▲ | ▲ | de Pedro et al. (2006) |
| | IP | 1000 ng/g | het: human | 10 days | ▲ | ▲ | ▲ | ▲ | ▲ | de Pedro et al. (2006) |
| | IP | 1000 ng/g | het: human | 8h | ▲ | ▲ | ▲ | ▲ | ▲ | de Pedro et al. (2006) |
| | IP | 1–100 ng/g | hom: leptin A1 | 2h | ▲ | ▲ | ▲ | ▲ | ▲ | Vivas et al. (2011) |
| | IP | 1–100 ng/g | hom: leptin A1 | 2h | ▲ | ▲ | ▲ | ▲ | ▲ | Yan et al. (2016) |
| | IP | 100 ng/g | hom: leptin A1 | 2h | ▲ | ▲ | ▲ | ▲ | ▲ | Yan et al. (2016) |
| | IP | 100 ng/g | hom: leptin A1 | 2h | ▲ | ▲ | ▲ | ▲ | ▲ | Yan et al. (2016) |
| Grass carp | IP | 2100 ng/g | hom | 1–13 days | ▲ | ▲ | ▲ | ▲ | ▲ | Li et al. (2010) |
| Green sunfish | IP daily | 200–400 ng/g | het: murine | 2 weeks | ▲ | ▲ | ▲ | ▲ | ▲ | Londraville and Duvall (2002) |
| Medaka | lepr KO | | | | | | | | | Chisada et al. (2014) |
| Mandarin fish | IP | 100–1500 ng/g | hom: leptin A | 2–4h | ▲ | ▲ | ▲ | ▲ | ▲ | Yuan et al. (2020) |
| | IP | 100–1500 ng/g | hom: leptin B | 2–4h | ▲ | ▲ | ▲ | ▲ | ▲ | Yuan et al. (2020) |
| Nile tilapia | IP | 400 ng/g | hom: leptin A1 | 2h | ▲ | ▲ | ▲ | ▲ | ▲ | Liu et al. (2018) |
| Rainbow trout | IVC | 0.5–5 ng/g | hom | 2h | ▲ | ▲ | ▲ | ▲ | ▲ | Gong et al. (2016) |
| | IVC | 5 ng/g | het: human | 6–24h | ▲ | ▲ | ▲ | ▲ | ▲ | Aguilar et al. (2010) |
| | IP | 720 ng/g | hom | 1–4h | ▲ | ▲ | ▲ | ▲ | ▲ | Murashita et al. (2008) |
| | In vitro | 10 nM | hom | 1h | ▲ | ▲ | ▲ | ▲ | ▲ | Aguilar et al. (2011) |
| | In vitro | 50 nM | hom | 1–3h | ▲ | ▲ | ▲ | ▲ | ▲ | Aguilar et al. (2011) |
| Striped bass | lepa KO | 100–1000 ng/g | het: human | 2–8h | ▲ | ▲ | ▲ | ▲ | ▲ | Won et al. (2012) |
| Zebrafish | lepr KO | | | | | | | | | Audira et al. (2018) |
| | lepr KO | | | | | | | | | Ahl et al. (2019) |

↑, increase; ▲, strong increase; ↓, decrease; ▼, strong decrease; ≈, no changes; IP, intraperitoneal; IVC, intracerebroventricular.
the rainbow trout hypothalamus (Aguilar et al., 2011). Considering this background in fish, and the well characterized role of leptin in glucose homeostasis in mammals (Denroche et al., 2012), it appears that the regulation of components of glucose homeostasis is a conserved function of leptin in fish and mammals. In contrast, the role of leptin in lipid homeostasis would have appeared later in mammals or have been lost and supplanted by an unknown pathway in fish (Michel et al., 2016). In any case, either responding to lipid and/or to glucose, leptin would be involved in signalling under metabolic conditions reflecting energy storage.

In mammals, the main effects of increased levels of leptin are a decrease in food intake and an increase in energy expenditure (Friedman, 2019). In teleost fish, several studies assessed the impact of leptin treatment on food intake in different species as summarized in Table 2. In general, most of these studies support an anorexigenic action for leptin in fish (Fig. 3), as that exerted in mammals. The clearest evidence for such a role for leptin derives from studies using homologous leptins. As far as we are aware, five studies to date assessed the short-term effect on food intake of intraperitoneal (IP) administration of homologous leptin with doses in the range of hundreds of ng/g. These include studies in goldfish (Yan et al., 2016), grass carp (Li et al., 2010), Nile tilapia (Liu et al., 2018), mandarin fish (Yuan et al., 2020), and rainbow trout (Murashtia et al., 2008). In these studies, a clear reduction in food intake occurred in response to the hormonal treatment. In the case of rainbow trout, an additional study using homologous leptin was carried out assessing its response upon intracerebroventricular (ICV) administration, also resulting in decreased food intake at 2 h post-administration (Gong et al., 2016). These results are in most cases comparable to those observed in the same species after short-term treatment with different heterologous mammalian hormones, as is the case of rainbow trout after ICV treatment (Aguilar et al., 2010) and goldfish after IP (de Pedro et al., 2006; Vivas et al., 2011; Volkoff et al., 2003) or ICV (Volkoff et al., 2003) treatments. However, in goldfish, other studies showed no effect of heterologous leptins on food intake upon short-term ICV treatment (de Pedro et al., 2006; Volkoff and Peter, 2001), which might relate to the different source of heterologous hormone and doses used. In other fish species, only studies using heterologous leptins are available. In these cases, while no effects were observed after ICV treatment in channel catfish (Silverstein and Plisetskaya, 2000), a decrease in food intake levels was reported after IP treatment in striped bass (Won et al., 2012). In the long-term (days), few studies assessed the effects of homologous or heterologous leptins. Those studies reflect decreased food intake after 10 days of IP treatment in goldfish (de Pedro et al., 2006) and no changes in green sunfish (Londraville and Duvall, 2002) and grass carp (Li et al., 2010). Further support to the anorectic role of leptin in fish comes from one study in medaka describing increased food intake in lepr KO fish compared to WT fish (Chisada et al., 2014). However, Michel and coworkers reported a lack of hyperphagia in adult zebrafish lacking a functional leptin receptor (Michel et al., 2016). Despite most of the available literature in fish argues in favour of an anorexigenic role of leptin (comparable to that of mammals), this assertion must be considered with caution due to several limitations. First, the number of species assessed is limited and belongs to a reduced number of fish groups (e.g., salmonids, cyprinids), thus not representing the high variability within this animal group. Second, as set out in earlier sections, different fish species may have a different number of leptins, which hampers comparisons among studies, since, for instance, modern studies assessing the impact of a specific leptin are difficult to compare with former studies in which the type of leptin assessed is not clear. Third, as just described, many studies use a leptin peptide of heterologous nature, mostly from mammalian sources, for experimentation. Despite the fact that the core of the molecule involved in binding to the receptor and eliciting a response is similar between fish and mammals, the rest of the sequence shows a high degree of divergence between both groups, which can lead to the observation of unexpected or incorrect results. Fourth, the administration pathways (IP, ICV, etc.), doses and response time assessed differ among studies, making comparisons difficult.

Changes in food intake levels generally relate to changes in the expression of hypothalamic neuropeptides involved in food intake regulation (Fig. 3). In mammals, it is well known that leptin exerts its anorexigenic response by acting in the hypothalamus (Schwartz et al., 2000). This occurs through inhibition the expression of NPY and agouti-related protein (AgRP) and the stimulation of proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) expression (Cowley et al., 2001; Baver et al., 2014). In fish, the homeostatic regulation of food intake is also based on the hypothalamic integration of metabolic, endocrine, and circadian information (Delgado et al., 2017) with mechanisms comparable but not identical (Soengas et al., 2018) to those known in mammals. The number of studies in fish assessing changes in hypothalamic neuropeptides after leptin treatment is limited, as shown in Table 2. Following the mammalian model (Schwartz et al., 2000), decreased mRNA levels of the orexins nyp and agpr are expected in the hypothalamus after leptin treatment. Such a decrease in nyp was reported in the hypothalamus of Nile tilapia (Liu et al., 2018), goldfish (Volkoff et al., 2003; Yan et al., 2016) and rainbow trout (Aguilar et al., 2011; Murashita et al., 2008), and in the brain of mandarin fish (Yuan et al., 2020). In other studies, however, no changes in hypothalamic nyp expression were observed in goldfish (de Pedro et al., 2006) and rainbow trout (Gong et al., 2016). As for agpr, a leptin-induced decrease in its expression has been reported in the hypothalamus of goldfish (Yan et al., 2016) and in the brain of mandarin fish (Yuan et al., 2020). Regarding the anorexins POMC and CART, the mRNA abundance of pomca increased in the hypothalamus of goldfish (Yan et al., 2016) and rainbow trout (Gong et al., 2016; Murashita et al., 2008) after IP or ICV leptin treatments. A similar leptin-induced increase in mRNA levels occurred for cartpt in the hypothalamus of goldfish (Volkoff et al., 2003; Yan et al., 2016) and rainbow trout (Gong et al., 2016) in vivo. However, no changes in the expression of either POMC or CART were detected in hypothalamus after leptin treatment in vivo in the later species (Aguilar et al., 2011). Conversely, both pomc and cart mRNAs decreased upon leptin treatment in the brain of mandarin fish (Yuan et al., 2020). While the number of studies assessing the impact of leptin treatment on hypothalamic neuropeptides is very limited, several important features are evident. Thus, in general, changes observed support an anorexigenic response of leptin due to the simultaneous raise in the expression of anorexigenic neuropeptides and the decrease in orexigenic neuropeptides. Furthermore, in all the studies reporting leptin-induced changes in expression of hypothalamic, decreased food intake levels occurred in response to the hormone, reinforcing the hypothalamic nature of changes involved in the anorectic effects of leptin. A further support to the involvement of hypothalamic neuropeptides in the anorectic role of leptin in fish comes from the findings obtained in KO fish. Thus, lepa KO zebrafish have higher agpr levels in brain than WT fish (Audira et al., 2018). Likewise, increased agpr mRNAs, together with decreased mRNA abundance of pomca and cartpt, were found in the brain of legr KO zebrafish compared to WT (Ahi et al., 2019). Finally, a raise in agpr mRNA abundance was also evident in the hypothalamus of medaka lepr KO compared to WT (Chisada et al., 2014). While hypothalamic neuropeptides play a major part in mediating leptin’s action in food intake, we cannot discard that besides a direct action of leptin on hypothalamic neurons producing neuropeptides, at least part of such role of leptin might relate to its effect on other hormones involved in the regulation of food intake. Thus, for instance, central treatment of leptin in goldfish promotes the satiating effects of cholecystokinin (CCK) and increased ccf mRNA abundance in hypothalamus, suggesting a synergistic action of both hormones (Volkoff et al., 2003). On the other hand, lepa KO zebrafish were observed to have higher ghrelin mRNA levels in brain than WT fish (Audira et al., 2018). These studies point towards a putative role of (at least) CCK and ghrelin as mediators of leptin’s anorexigenic action. Nevertheless, further studies are required to delve into these few observations.
5. Conclusions/Perspectives

This work reviews the current knowledge on the role of leptin in food intake regulation in teleost fish. When comparing leptin physiology in teleost fish with that of mammalian models several difficulties arise. One important limitation comes from the fact that teleost fish is a diverse group comprising 40 orders. This huge diversity is not enough covered by studies of leptin physiology, which are represented by studies performed in species from 11 of those orders, of which some of the main groups are not included. Thus, most of the studies assessed the relatively old orders Salmoniformes and Cypriniformes as well as the relatively modern order Perciformes. However, studies on species from other highly representative orders (in terms of number of species) are scarce (e.g., Characiformes, Pleuronectiformes, Tetraodontiformes) or inexist ent (e.g., Gobiiformes, Spariformes). Therefore, caution must be taken when making generalizations from species of the groups assessed to the others. Second, the additional rounds of genome duplication in teleost fish compared with mammals resulted, in some fish species, in the presence of more than one lept paralogue. While fish leptin functions appear to share similarities with their mammalian orthologues, some differences exist, and in those fish species having two leptins, the specific functions and site of action of each of them are not clear, suggesting a more complex picture than that known in mammals. A third important limitation is the high diversity of dietary habits of fish species either in terms of main food components (herbivorous, omnivorous, and carnivorous) or in the way of feeding (continuous feeders, predatory), which results in a highly heterogeneous group. This fact probably explains some of the differences observed in leptin physiology between fish and mammals. Thus, mammalian models typically used in studies on leptin physiology (i.e. rodents) are mostly omnivorous while fish models assessed include herbivorous, omnivorous and carnivorous species. The differences observed between fish and mammals in the response patterns of leptin to the absence/presence of food might relate to such different dietary habits. In this regard, the response of decreased leptin activity to conditions of food deprivation observed in some fish studies, which is comparable to that known in mammals, occurred mostly in omnivorous and herbivorous species. In contrast, a higher leptin activity in response to fasting occurred mostly in carnivorous fish species. We can speculate that this finding relates to the fact that carnivorous species face frequent periods of food deprivation due to their predatory nature compared with omnivorous and herbivorous fish species that are continuous feeders. In a similar way, the effects of feeding on leptin physiology in fish seem to follow a response pattern characterized by a comparable response to that of mammals in the case of omnivorous/herbivorous species while an opposite response usually occurred in carnivorous species. Furthermore, in those cases in which the response of leptin to fasting or feeding were evaluated in the same species, opposite effects of fasting and feeding were found on most occasions. These include rainbow trout (Kling et al., 2009; Gong et al., 2013; Johansson and Björnsson, 2015), striped bass (Wen et al., 2012), chub mackerel (Ohga et al., 2015), and fine flounder (Fuentes et al., 2012). It is interesting to mention that all those species are carnivorous whereas in the cases in which opposite effects of fasting and feeding were not found this mostly occurred in omnivorous/herbivorous species such as goldfish (Trincoc et al., 2012), grass carp (Gong et al., 2017; Liang et al., 2019b) or common carp (Ruisong et al., 2006a).

Based on all the information gathered in this review, it seems reasonable to conclude that the main tissue involved in leptin synthesis in teleost fish is the liver, compared with the adipose tissue in mammals. However, we must consider that leptin expression is present in several tissues in most of the fish species assessed. The physiological significance of this synthesis in different tissues (if any) or the contribution of leptin synthesis in these tissues to circulating leptin levels are not clear and need further investigation. What seems clear is that leptin synthesis in teleost fish is more dependent on environmental variables compared to mammals. This is not surprising considering that water surrounds fish and conditionates their lives. We do not know, however, how these factors impact on leptin system precisely, as only few studies addressed this topic. The effects of dietary composition on leptin physiology in fish seem also clear, suggesting, in general, that enhanced availability of nutrients in the diets, especially lipids and carbohydrates, elicits an activation of the leptin system. However, leptin in fish, in contrast to mammals, is more likely acting as a glucostat rather than a lipostat (Michel et al., 2016). This activation of the leptin system in response to enhanced availability of nutrients seems reasonable considering that such a situation generally involves the inhibition of food intake in fish (Conde-Sieira and Soengas, 2017) and that leptin is anorectic. However, this anorectic effect of nutrients needs to be fitted with the general decrease of leptin function in carnivorous (not omnivorous) fish species. Unfortunately, no studies attempted yet to evaluate the impact of different protein/amino acid levels on leptin synthesis. This is presumably highly important considering the use of amino acids as energy fuel in carnivorous fish species.

Available studies regarding the impact of leptin treatment on food intake regulation in fish are limited, and most of them carried out with heterogeneous hormones. However, a general anorectic effect of leptin is apparent in fish, in a way comparable to that observed in mammals, suggesting a more or less conserved function for leptin throughout vertebrate evolution. This function would involve comparable changes in the expression of hypothalamic neuropeptides related to the hypothalamic integration of food intake response. However, such anorectic response is striking considering, as commented above, that only in a few species (mostly herbivorous and omnivorous) the synthesis of leptin occurs in conditions of feeding and food deprivation comparable to those in mammals. What is the logical of inducing an anorectic response in carnivorous fish species similar to that of mammals when the presence/absence of food elicits opposed responses in the leptin system? The answer to this question does not probably relies on a single cause but is likely the result of an interaction between different factors, of which dietary habits, the presence of different leptin paralogues, and different function depending on tissues, among others, may be important.

In conclusion, the leptin system in teleost fish shares several similarities, but also important differences, in terms of gene structure, synthesis and functionality with that of mammals. Some aspects, however, are even difficult to determine whether are similar or different between groups due to the almost inexist studies carried out in teleost fish, as are, for instance, the mechanisms involved in leptin signalling or the hypothalamic integration of leptin and other metabolic and endocrine signals. It is also not clear if different mechanisms of binding and activation of receptors are present in fish species compared with mammals as well as the mechanisms involved in leptin signalling since only few studies carried out in only one species (rainbow trout) are available. Clearly, more studies are required to know the precise mechanisms of leptin functioning in teleost fish, but certainly assessing leptin physiology in these vertebrates offers a more complex field of study that the more homogeneous mammals studied so far.

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Declaration of competing interest

None.
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