© 2021. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2021) 224, jeb238790. doi:10.1242/jeb.238790

REVIEW

Functional significance and physiological regulation of essential trace metals in fish

Theanuga Chandrapalan and Raymond W. M. Kwong*

ABSTRACT

Trace metals such as iron, copper, zinc and manganese play essential roles in various biological processes in fish, including development, energy metabolism and immune response. At embryonic stages, fish obtain essential metals primarily from the yolk, whereas in later life stages (i.e. juvenile and adult), the gastrointestine and the gill are the major sites for the acquisition of trace metals. On a molecular level, the absorption of metals is thought to occur at least in part via specific metal ion transporters, including the divalent metal transporter-1 (DMT1), copper transporter-1 (CTR1), and Zrt- and Irt-like proteins (ZIP). A variety of other proteins are also involved in maintaining cellular and systemic metal homeostasis. Interestingly, the expression and function of these metal transport- and metabolism-related proteins can be influenced by a range of trace metals and major ions. Increasing evidence also demonstrates an interplay between the gastrointestine and the gill for the regulation of trace metal absorption. Therefore, there is a complex network of regulatory and compensatory mechanisms involved in maintaining trace metal balance. Yet, an array of factors is known to influence metal metabolism in fish, such as hormonal status and environmental changes. In this Review, we summarize the physiological significance of iron, copper, zinc and manganese, and discuss the current state of knowledge on the mechanisms underlying transepithelial metal ion transport, metal–metal interactions, and cellular and systemic handling of these metals in fish. Finally, we identify knowledge gaps in the regulation of metal homeostasis and discuss potential future research directions.

KEY WORDS: Iron, Copper, Zinc, Manganese, Trace metal homeostasis, DMT1, CTR1, ZIP, Fish

Introduction

Essential trace metals such as iron, copper, zinc and manganese are vital components for a multitude of biochemical reactions and act as cofactors for various enzymes. They can be found in proteins such as the oxygen-transporting hemoproteins (iron as a cofactor), iron–sulfur clusters involved in DNA synthesis, cytochrome *c* oxidase of the electron transport chain (copper) and superoxide dismutase (SOD; copper, zinc, manganese) of the antioxidant defense system (Dalziel et al., 2006; de Souza and Bonilla-Rodriguez, 2007; Harris, 1992; Puig et al., 2017). In fish, trace metal deficiency results in impairment to health and physiological performance (Clearwater et al., 2002; Makwinja and Geremew, 2020; Song et al., 2017). On the contrary, these essential metals can become detrimental when in excess, often as a result of increased oxidative stress and induced pathophysiological conditions (Wood et al., 2011). Hence, it is important to tightly regulate and maintain trace metal levels within physiologically safe concentrations.

In adult fish, the acquisition of trace metals occurs via the gill and the gastrointestine (Bury and Glover, 2003). The precise molecular mechanisms regulating the epithelial transport and metabolism of essential metals in fish have remained an active research question, as most of our current understanding is primarily based on mammalian studies. Despite this knowledge gap, recent research has provided new information on the biological significance, physiological regulation and functional involvement of various metal regulatory proteins in fish. These research efforts have demonstrated the complex nature of the regulatory and compensatory mechanisms involved in the maintenance of trace metal balance in fish. Additionally, several essential trace metals have been shown to interact at multiple biological levels (e.g. metal transporters, metal-binding proteins, metal-regulatory signalling pathways). Therefore, an alteration in the homeostasis of one trace metal may have the potential to affect the regulation of several other metals. Moreover, a variety of factors have been shown to influence the way fish regulate metal metabolism and respond to changes in metal availability. These factors include, for example, developmental stage, dietary composition and environmental perturbations (Bury and Glover, 2003; Chandrapalan and Kwong, 2020; Glover et al., 2016; Long et al., 2015).

The uptake and metabolism of essential trace metals in fish have been previously reviewed in several articles and book chapters (Bury and Glover, 2003; Wood et al., 2011; Zhao et al., 2014). In the present Review, we provide an overview of the current state of knowledge on the physiological function of essential trace metals in fish, with a focus on iron, copper, zinc and manganese. We also discuss the interactions between metals (e.g. epithelial transport, transporter expression, cellular and systemic handling), the mechanisms underlying their regulation and recent progress in these topics. Finally, we identify knowledge gaps and discuss potential future research directions.

Physiological significance of essential trace metals in fish

Essentiality in development

Iron, copper, zinc and manganese are found in an array of metalloproteins and are cofactors for enzymes essential in various physiological functions, including oxygen transport, antioxidant activity, metal storage, and metabolism and redox balance (Table 1). Before the onset of exogenous feeding, fish embryos obtain nutrients, including essential metals, primarily from the maternally derived yolk stores via the yolk syncytial layer (Carvalho and Heisenberg, 2010; Fraenkel et al., 2005; Riggio et al., 2003; Thomason et al., 2017). Parental deficiency in essential metals such as zinc can have adverse effects on the offspring, including elevated mortality, decreased activity and possible alterations in epigenetic regulators (DNA methyltransferases) (Beaver et al.,

Department of Biology, York University, Toronto, Ontario, M3J 1P3, Canada.

*Author for correspondence (rwmkwong@yorku.ca)

R.W.M.K., 0000-0002-0784-9651

Journal of Experimental Biology (2021) 224, jeb238790. doi:10.1242/jeb.238790

List of symbols and abbreviations

ASIC	acid-sensing ion channel
ATOX1	antioxidant 1 copper chaperone
CCS	copper chaperone for superoxide dismutase
COX17	cytochrome *c* oxidase copper chaperone
CRISPR	clustered regularly interspaced short palindromic repeats
CTR1	copper transporter-1
Dcytb	duodenal cytochrome b
DHA	docosahexaenoic acid
DMT1	divalent metal transporter-1
ECaC	epithelial calcium channel
ENaC	epithelial sodium channel
EPA	eicosapentaenoic acid
GIT	gastrointestinal tract
HIF	hypoxia-inducible factor
IRE	iron-responsive element
IRP	iron-regulatory protein
LEAP-1	liver-expressed antimicrobial peptide
MT	metallothionein
MTF1	metal-responsive transcription factor-1
ROS	reactive oxygen species
SOD	superoxide dismutase
T_3	triiodothyronine
U_{crit}	critical swimming speed
$\dot{V}_{O_2,max}$	maximum rate of oxygen consumption
ZIP	Zrt- and Irt-like protein
ZnT	zinc transporter

2017). In vertebrates, iron is known to be essential for the development of the brain, including neurocognitive development and neurotransmitter metabolism (McCann et al., 2020; Wang et al., 2019). In zebrafish (*Danio rerio*) embryos, both high levels of iron and copper are deposited in the yolk syncytial layer (Bourassa et al., 2014). High levels of iron are also present in the brain and tail of developing zebrafish (Bourassa et al., 2014). The head also appeared to be the major region of iron deposition in larval zebrafish exposed to iron, leading to a disruption in neurobehavioral performance (Hassan and Kwong, 2020). Similarly, dysregulation of manganese balance in the brain is found to adversely affect neurosensory function in larval zebrafish (Bakthavatsalam et al., 2014; Xia et al., 2017). These results suggest that the maintenance of trace metal balance in the brain is of critical importance during periods of neurodevelopment. Likewise, a few previous studies have shown that zinc is involved in embryogenesis in fish. For example, morpholino-mediated gene knockdown of the zinc transporter ZIP7 resulted in morphological defects and reduced zinc concentrations in the brain, eyes and gills of larval zebrafish (Yan et al., 2012). Additionally, zinc was found to be highly deposited in the hatching gland cells of larval zebrafish, and knockdown of ZIP10 resulted in an impairment in the development of the hatching gland, leading to failure in hatching (Muraina et al., 2020). These results indicate the essential role of zinc and specific zinc transporters in the early development of fish.

Recent studies have shown that iron is involved in bone formation signalling pathways and that feeding with an iron-enriched diet can increase the standard body length of developing zebrafish (Bo et al., 2016; Chandrapalan and Kwong, 2020). Similarly, copper is found to be essential for notochord development in zebrafish, primarily owing to its role in the enzyme lysyl oxidase, which promotes elastin–collagen crosslinking in the notochord (Gansner et al., 2007; Mendelsohn et al., 2006). Copper is also important in the formation of pigmentation in zebrafish through its involvement in the copper-dependent melanogenic enzyme tyrosinase (Ishizaki et al., 2010). Additionally, supplementation of diets with manganese and zinc has been shown to improve survival, bone mineralization and skeletal formation in the post-larvae of Senegalese sole (*Solea senegalensis*) (Viegas et al., 2021). In another study with post-smolt Atlantic salmon (*Salmo salar*), however, a change in dietary manganese level (5–65 mg kg^{-1}) was found to have no effect on specific growth rate over an 8-week experimental period (Antony Jesu Prabhu et al., 2019). In addition to possible species-specific differences in dietary metal requirements, differences in the developmental stages and dietary composition used across studies may also account for some of these mixed results.

Current understanding of the nutritional requirement for most essential metals is limited to a few fishes, particularly commercially important taxa such as carps, yellow catfish, groupers and salmonids (Chanda et al., 2015; Davis and Gatlin, 1996; Watanabe et al., 1997). For example, a range of 30–170 mg iron, 15–40 mg zinc, 2–20 mg manganese and 1–5 mg copper (per kg of diet) is proposed to be the minimal requirement for the maintenance of fish health in aquaculture practice (Watanabe et al., 1997). However, it should be noted that most of these studies were conducted with metal salts added in formulated diets, whereas biologically incorporated metals such as those found in prey and natural fish feeds are generally more bioavailable (Deforest and Meyer, 2015; Mebane et al., 2020). Therefore, the actual nutritional requirements for dietary metals in feral fish are likely to be lower than those estimated using formulated diets. Assessing the dietary requirements of metals with natural live diets would be invaluable to better understand how fish regulate metal absorption and balance during periods of metal deficiency or overload.

Energy metabolism

In vertebrates, iron is essential in the synthesis of oxygen transport proteins (e.g. hemoglobin and myoglobin) and enzymes involved in electron transfer and oxidation–reduction (Ganz, 2013). Manganese and copper are also known to be involved in glucose metabolism and mitochondrial functioning (Baker et al., 2017; Li and Yang, 2018). Therefore, these metals play an essential role in energy metabolism. A few studies have demonstrated that iron and copper status are also linked to the energetic parameters of fish. For example, lower critical swim speeds (U_{crit}) and reduced maximal oxygen uptake ($\dot{V}_{O_2,max}$) values were observed in rainbow trout (*Oncorhynchus mykiss*) experiencing anemia (i.e. through blood removal) (Gallaugher et al., 1995). In yellow catfish (*Pelteobagrus fulvidraco*), changes in dietary copper levels were found to alter lipogenic enzyme activities and lipid metabolism (Chen et al., 2015). Additionally, dietary supplementation of iron or copper was found to improve hematological parameters in Siberian sturgeon (*Acipenser baerii*) and stinging catfish (*Heteropneustes fossilis*), including increases in hemoglobin content, erythrocyte count and hematocrit value (Moazenzadeh et al., 2020; Zafar and Khan, 2020). These enhancements are thought to increase oxygen transport capabilities in fish.

Immune function

Hepcidin (previously termed as liver-expressed antimicrobial peptide; LEAP-1) is a peptide hormone that regulates intestinal iron uptake and is associated with innate immune signalling (Drakesmith and Prentice, 2012). The first study that demonstrated the potential role of hepcidin in the immune response of fish was conducted in white bass (*Morone chrysops*), which showed that an

Table 1. An overview of the function of metalloproteins and their associated metal co-factors

Protein/enzyme	Function	Metal/co-factor
Oxygen transport		
Hemoglobin	Oxygen binding protein found in red blood cells	Fe
Myoglobin	Oxygen binding protein found primarily in muscles	Fe
Antioxidant activity		
Catalase	Catalyzes the decomposition of hydrogen peroxide	Fe, Cu, Mn, Zn
Peroxidase	Catalyzes the decomposition of peroxides	Fe
Superoxide dismutase	Catalyzes the dismutation of superoxide radicals	Cu, Mn, Zn
Metal storage and metabolism		
Ferritin	Intracellular iron storage	Fe
Transferrin	Iron transport in the blood	Fe
Metallothionein	Zinc transport, storage and detoxification	Cu, Zn
Ceruloplasmin	Copper transport to tissues	Cu
Hephaestin	Ferroxidase for the conversion of Fe^{2+} to Fe^{3+}	Cu
Ferric reductase	Reduces Fe^{3+} to Fe^{2+}	Cu
Other metabolic and redox activities		
Cytochromes and NADH dehydrogenase	Involved in the electron transport chain and cellular respiration	Fe, Cu
Glutamine synthetase	Catalyzes glutamine synthesis from glutamate	Mn
Pyruvate carboxylase	Catalyzes the carboxylation of pyruvate	Mn
Lipase	Breaks down fats	Mn
Tyrosinase	Catalyzes the production of pigments	Cu

NADH, nicotinamide adenine dinucleotide.

infection with a pathogen in this fish species resulted in a 4500-fold induction of hepcidin mRNA expression (Shike et al., 2002). Since this pioneering study, increasing evidence has indicated a close association between hepcidin and iron status in immune function across many fish species (Cuesta et al., 2008; Shi and Camus, 2006; Tarifeño-Saldivia et al., 2018). Nevertheless, how hepcidin orchestrates its dual functional roles in iron regulation and immune response in fish has remained unclear. In zebrafish, it was found that intraperitoneal injection of bacterial DNA can stimulate hepatic hepcidin expression and reduce serum iron levels (Jiang et al., 2017). The shift from circulating iron to intracellular storage was suggested to reduce the availability of iron to invading microorganisms, thereby enhancing antibacterial immunity (Drakesmith and Prentice, 2012). Several studies have also shown that the immune response of fish can be influenced by dietary metals. For example, iron deficiency has been shown to impair the immune function of grass carp (*Ctenopharyngodon idella*) by reducing the content of immunoglobulin and the transcript abundance of various anti-inflammatory cytokines (Guo et al., 2018a). Juvenile Siberian sturgeon fed on a copper-enriched diet also exhibit an increase and a decrease in the levels of neutrophil and lymphocyte, respectively (Moazenzadeh et al., 2020). In mammals, zinc is involved in various aspects of the immune function, including activation of immune cell signalling and stimulation of the development of immune cells (Prasad, 2008; Wessels et al., 2017). A few previous studies have also shown that zinc promotes immune functioning in fish. For example, feeding of iridescent shark (*Pangasianodon hypophthalmus*) with a zinc-enriched diet has been found to enhance the immune-hematological status and improve their survival rate after pathogen infection (Kumar et al., 2017). Likewise, grass carp (*Ctenopharyngodon idella*) fed on a zinc-deficient diet have been shown to exhibit an impairement in enteristis resistance (Song et al., 2017). The role of manganese in immune response in fish is less understood, but exposure to high levels of waterborne manganese has been shown to alter the number of immune cells and the level of antimicrobial enzymes (Aliko et al., 2018; Do et al., 2019; Zafar and Khan, 2019).

Increasing evidence has also suggested that the iron-binding proteins ferritin and transferrin are responsive to bacterial infection in fish. For example, in rock cod (*Eleginops maclovinus*), bacterial infection increases the mRNA abundance of the ferritin-H subunit (Martínez et al., 2017). In Japanese flounder (*Paralichthys olivaceus*) and rock bream (*Oplegnathus fasciatus*), bacterial infection increases the mRNA expression of the ferritin-M subunit (Elvitigala et al., 2013; Wang and Sun, 2015). Additionally, both ferritin and transferrin expression are modulated in sea bass (*Dicentrarchus labrax*) during bacterial infection, with the levels of ferritin increased in both the liver and the brain (Neves et al., 2009). An increase in transferrin expression was also observed in common carp (*Cyprinus carpio*) during blood parasite infection (Kamińska-Gibas et al., 2020). The induction of iron-binding proteins during infection is proposed to be an acute inflammatory response to sequester iron and thereby reduce its availability for microbial or pathogen proliferation in the host (Ong et al., 2006). Alternatively, when excess iron is accumulated in macrophages, it can result in the production of free radicals via the Fenton reaction and subsequently aid in the elimination of the intruding pathogen (Tarifeño-Saldivia et al., 2018).

Homeostatic regulation of essential trace metals

Regulation of transepithelial metal uptake

During early developmental stages, before the gills and the gastrointestinal tract (GIT) are fully developed, the uptake of essential trace metals from the environment can occur through the skin. Several studies have shown that larval fish can acquire and accumulate essential metals from the water (Guo et al., 2016; Hassan and Kwong, 2020; Puar et al., 2020). However, the precise pathways for their uptake remain unclear. A few previous studies with the zebrafish model have shown that several specific metal transporters, including those mediating the uptake of iron or zinc, do not appear to be expressed in the skin during early development (Donovan et al., 2002; Haller et al., 2018; Puar et al., 2020). Nevertheless, the epithelial calcium (Ca^{2+}) channel (ECaC), which is expressed in the skin of larval fish (Kwong et al., 2014; Lin and Hwang, 2016; Pan et al., 2005), can transport not only calcium but also iron and zinc (Qiu and Hogstrand, 2004). In contrast, the uptake of copper has been found to be sodium sensitive and is proposed to occur via sodium transporting channels (Grosell and Wood, 2002;

Handy et al., 2002). Although the epithelial sodium channels (ENaCs) were considered a potential candidate in previous studies, current genomic data suggest that ENaCs are lost in the actinopterygian lineage and thus absent in teleost fish (Wichmann and Althaus, 2020). Instead, it has been proposed that acid-sensing ion channels (ASICs), which have been documented in other vertebrates for copper transport, are responsible for the sodium-dependent copper transport in fish. Indeed, ASICs are expressed in several fish species including rainbow trout and zebrafish (Dymowska et al., 2015; Zimmer et al., 2018); however, whether ASICs can in fact transport copper awaits investigation. Overall, it seems possible that the waterborne uptake of trace metals during the early developmental stages is mediated by ion transporters (i.e. via ionocytes) expressed in the skin. Interestingly, the skin is also suggested to be an important site for the absorption of iron in adult Pacific hagfish (*Eptatretus stoutii*) (Glover et al., 2016), although the molecular pathways that mediate iron uptake in this species are yet to be determined.

In juvenile and adult fish, the acquisition of essential trace metals occurs via the gill and the GIT. Despite the capability of the gill to absorb trace metals from water, the diet is thought to be the major source of essential metals in fish (Bury and Glover, 2003; Watanabe et al., 1997). This is because in clean environments, the concentration of essential metals is generally quite low and at circumneutral pH, some of these metals such as iron may precipitate and form insoluble metal oxides and hydroxides. A previous study with the marine teleost *Psetta maxima* and elasmobranch *Scyliorhinus canicular* has shown that over 60% of the uptake of essential metals, including manganese, cobalt and zinc, occurs via the dietary route (Mathews and Fisher, 2009). In the GIT, the predominant region for the absorption of essential metals varies for different metals and among species. For example, iron absorption occurs primarily in the posterior region of the GIT in marine flounder (*Platichthys flesus*) (Bury et al., 2001) as opposed to the anterior region in freshwater rainbow trout (Kwong and Niyogi, 2009). Interestingly, the stomach is the major region for the absorption of copper in rainbow trout (Nadella et al., 2011). The mechanisms for the absorption of copper also appear to be different between the stomach and intestine, with the latter partially mediated by sodium- or iron-sensitive pathways (Nadella et al., 2007, 2011). In African walking catfish (*Clarias gariepinus*), copper uptake occurs predominantly in the distal intestine and is dependent on a chloride gradient (Handy et al., 2000). Furthermore, the levels of dietary trace metals and major ions (e.g. sodium, calcium, magnesium and potassium) have been shown to influence the acquisition of metals from water. For example, waterborne iron uptake was found to increase in zebrafish fed on an iron-deficient diet (Cooper et al., 2006a). Marine medaka (*Oryzias melastigma*) fed on a low-iron diet also exhibited an increase in waterborne iron uptake (Wang and Wang, 2016). Interestingly, in rainbow trout, it has been shown that increasing the levels of dietary sodium reduced copper acquisition via the gills (Pyle et al., 2003). Similarly, elevated dietary calcium levels have been found to reduce the branchial absorption of zinc in rainbow trout (Niyogi and Wood, 2006). These findings suggest that the levels of trace metals and major ions in the diet can modulate trace metal uptake at the gill. How fish coordinate metal uptake between the gill and the GIT requires further investigation.

Molecular machinery and metal-metal interactions

Iron in the diet primarily exists in the ferric form (Fe^{3+}) and is first converted to the ferrous form (Fe^{2+}) by ferric reductase (also called duodenal cytochrome b; Dcytb) before its absorption from the intestine via the divalent metal transporter-1 (DMT1). According to the NCBI database, the gene encoding for ferric reductase has been identified in various fish species, such as rainbow trout, medaka, Indian glassy fish (*Parambassis ranga*) and zebrafish. Additionally, the activity of ferric reductase has been detected in rainbow trout and the air-breathing fish *Anabas testudineus* (Carriquiriborde et al., 2003; Rejitha and Peter, 2013). In mammals, ferric reductase is also proposed to mediate the reduction of cupric ion (Cu^{2+}) to cuprous ion (Cu^{+}) before its absorption by the copper transporter CTR1 (Wyman et al., 2008). In fish, DMT1 is thought to be the major transporter for the acquisition of iron from the diet and water (Bury, 2003). Notably, zebrafish mutants that produced truncated *dmt1* mRNA developed hypochromic and microcytic anemia (Donovan et al., 2002), suggesting its essential role in maintaining iron homeostasis. The gene encoding for DMT1 has been detected in early life stages and various tissues in adults (Chandrapalan and Kwong, 2020; Craig et al., 2008; Donovan et al., 2002; Hassan and Kwong, 2020; Wang and Wang, 2016). Interestingly, both an increase and a decrease in *dmt1* expression levels following iron exposure have been reported (Chandrapalan and Kwong, 2020; Cooper et al., 2006a; Craig et al., 2008; Hassan and Kwong, 2020; Kwong et al., 2013; Wang and Wang, 2016). The changes in *dmt1* expression could be indirect effects induced by iron because the iron transport pathways are also involved in the absorption and homeostatic regulation of several other essential metals (e.g. compensation for other metals; discussed below).

In mammals, DMT1 is capable of transporting not only iron but also other divalent metals such as manganese and copper (Arredondo et al., 2003; Garrick et al., 2003). However, a few recent studies have argued that DMT1 is not physiologically important in the intestinal absorption of copper or manganese (Illing et al., 2012; Shawki et al., 2015). In fish, direct evidence that shows the substrate selectivity of piscine DMT1 is limited to iron and the nonessential metal cadmium (Cooper et al., 2007). Nevertheless, many studies have demonstrated the interactions between iron and other essential metals, such as copper, manganese and zinc (Chandrapalan and Kwong, 2020; Craig et al., 2008; Hassan and Kwong, 2020; Kwong and Niyogi, 2009; Nadella et al., 2007). These interactions were observed either at the uptake surfaces or in regulating internal metal balance. In mammals, copper and zinc were also found to affect DMT1 expression (Iyengar et al., 2009; Tennant et al., 2002; Yamaji et al., 2001), whereas calcium appeared to be a non-competitive inhibitor for iron absorption in the enterocytes (Shawki and Mackenzie, 2010; Thompson et al., 2010). In addition to DMT1, the apical uptake of manganese and copper can also be mediated by other transporters, such as the zinc transporters ZIP14 and CTR1 (Mackenzie et al., 2004; Tuschl et al., 2016; Zhao et al., 2014). In the stomach of rainbow trout, CTR1 is proposed to be the major transporter responsible for the absorption of copper (Nadella et al., 2011).

The basolateral extrusion of iron and copper from absorptive cells is known to be mediated by ferroportin and copper transporting ATPases (e.g. ATP7a), respectively (Donovan et al., 2000; Mendelsohn et al., 2006). Ferroportin is also thought to mediate the extrusion of manganese (Madejczyk and Ballatori, 2017; Seo and Wessling-Resnick, 2015). In a human colorectal adenocarcinoma cell line, Caco-2, copper has been shown to induce the expression of ferroportin (Tennant et al., 2002). These findings indicate that in addition to the apical surfaces (e.g. via DMT1), metal–metal interactions may also occur at the basolateral membrane, via either competition for export or modulation in the

expression of metal export proteins. Advances in gene editing also provide a useful tool to reveal the functional significance of metal transporters and related proteins (Table 2). A recent study with zebrafish larvae has demonstrated that CRISPR/Cas9-mediated knockout of the zinc transporter ZnT10, a putative manganese exporter, results in manganese overload and hypermanganesaemia-related phenotypes, which can be partially rescued by iron treatment (Xia et al., 2017). This finding suggests that the interaction between iron and manganese is likely to be competitive in nature. Moreover, feeding with different levels of dietary iron can modulate the whole-body content of zinc and mRNA expression levels of the zinc transporters ZIP8 and ZIP14 in developing zebrafish (Chandrapalan and Kwong, 2020). Interestingly, zebrafish experiencing a knockout of ZIP14, which is a major transporter for zinc, but also capable of transporting manganese and iron, were found to increase manganese accumulation without affecting zinc and iron balance in their body (Tuschl et al., 2016). This result suggests that ZIP14 is important in manganese clearance in the kidney and that there are additional transport pathways to regulate and maintain the homeostasis of zinc and iron. To conclude, trace metal interactions may occur during the uptake process, potentially via direct metal–metal competition for absorption and/or modulation in the expression of metal transport proteins (Fig. 1).

Cellular and systemic handling of essential trace metals

In vertebrates, including fish, ferritin and transferrin regulate intracellular iron storage and iron delivery to various tissues, respectively (Bury et al., 2011). Their levels and binding capacities are known to be induced by iron exposure in fish (Carriquiriborde et al., 2003; Kwong et al., 2013). Recent advances with size or subcellular fractionation have provided some new information on the distribution of trace metals among cytosolic proteins within a cell. For example, in the liver of European chub (*Squalius cephalus*), it was suggested that copper was predominantly associated with metallothionein and, to a lesser extent, carbonic anhydrase and SOD (Krasnići et al., 2013). Iron appeared to be primarily bound to ferritin, whereas manganese was associated with albumin and transferrin (Krasnići et al., 2013). These results suggest that in addition to their interactions during absorption (see above), trace metals may also compete for binding sites within a cell or in circulation.

The delivery of copper in the blood is primarily mediated by ceruloplasmin (Das and Sahoo, 2018). In mammals, ceruloplasmin is also known to play an important role in systemic iron handling by oxidizing ferrous iron into ferric iron to facilitate its binding to transferrin (Hellman and Gitlin, 2002). Ceruloplasmin has been identified in various fish species, including icefish (*Chionodraco rastrospinosus*), Indian carp (*Aeromonas hydrophila*) and channel catfish (*Ictalurus punctatus*) (Liu et al., 2011; Sahoo et al., 2013; Scudiero et al., 2007). The mRNA expression level of ceruloplasmin has also been found to increase in channel catfish experiencing bacterial infection and iron overload (Liu et al., 2011). Copper is transported into cells by copper transporters (e.g. CTR1 and DMT1), and within the cell, virtually all free copper is sequestered by metal binding proteins including the copper chaperones (Kaplan and Maryon, 2016; Rae et al., 1999). The copper chaperones perform the dual functions of intracellular copper storage and trafficking. These proteins include the antioxidant 1 copper chaperone (ATOX1) for transporting copper to copper-ATPase in the Golgi network, the cytoplasmic copper chaperone (also called copper chaperone for superoxide dismutase; CCS) for delivering copper to SOD, and cytochrome *c* oxidase copper chaperone (i.e. COX17) for delivering copper to cytochrome c oxidase in the mitochrondria (Cheng et al., 2017; Kwok and Chan, 2019; Leung et al., 2014).

Our current understanding of the signalling pathways (e.g. initiation of transcription, post-translational modifications) involved in trace metal regulation is primarily derived from studies from mammalian counterparts. Several iron transport- and metabolism-related proteins, including DMT1, ferroportin, ferritin and

Table 2. Application of forward and reverse genetics with the zebrafish (*Danio rerio*) model in understanding the functional involvement of metal transport- and metabolism-related proteins in development and in the regulation of iron, copper or manganese

Protein	Major substrate*	Findings	Reference
DMT1	Iron	DMT1-deficient fish experience microcytic anemia and hypochromic anemia, and have a decreased number of erythroid cells	Donovan et al., 2002; Grillo et al., 2017
FPN1	Iron	Required for Fe absorption and mutants develop hypochromic anemia	Donovan et al., 2000; Donovan et al., 2005
Frrs1b	Iron	Important for normal cellular and mitochondrial Fe metabolism	Xue et al., 2015
Tfa	Iron	Tfa is required for Fe transport from the yolk to the embryo and morphants displayed anemia	Fraenkel et al., 2009
TfR1	Iron	Knockdown of TfR1a produces anemia in embryos whereas knockdown of TfR1b does not	Fraenkel et al., 2009
TfR2	Iron	TfR2-deficient fish have reduced hepcidin expression but do not exhibit anemia nor morphological defects	Fraenkel et al., 2009
Mfrn	Iron	Import Fe into the mitochondria of developing erythroid cells	Shaw et al., 2006
CTR1	Copper	Mutant larvae exhibit pigmentation defects and mitochondrial Cu deficiency Embryonic lethality in morphants; neural tissue is most sensitive to *ctr1* loss	Soma et al., 2018; Mackenzie et al., 2004
ATP7a	Copper	Involved in notochord development and pigmentation in zebrafish	Mendelsohn et al., 2006
ATP7b	Copper	Mutants exhibit increased Cu accumulation, histopathology in the liver, behavioral impairment and defects in central nervous system myelination	Mi et al., 2020; Zhang et al., 2020
ZnT10	Zinc	Mutant larvae exhibit impaired Mn efflux and increased Mn accumulation in the brain and liver; mutants develop neurological deficits in adulthood	Xia et al., 2017
ZIP8	Zinc	Mutants develop idiopathic scoliosis-like phenotypes and display abnormal vertebral development, delayed growth and decreased motor activity	Haller et al., 2018
ZIP14	Zinc	Mutants develop Mn overload and impaired locomotor activity.	Tuschl et al., 2016

*Note that some of the metal transporters are known to transport multiple metals (see section 'Homeostatic regulation of essential trace metals'). DMT1, divalent metal transporter-1; FPN1, ferroportin-1; Frrs, ferric reductase; Tf, transferrin; TfR, transferrin receptor; Mfrn, mitoferrin; CTR1, copper transporter-1; ATP7, copper transporting ATPase; ZnT, zinc transporter; ZIP, Zrt- and Irt-like protein.

Journal of Experimental Biology (2021) 224, jeb238790. doi:10.1242/jeb.238790

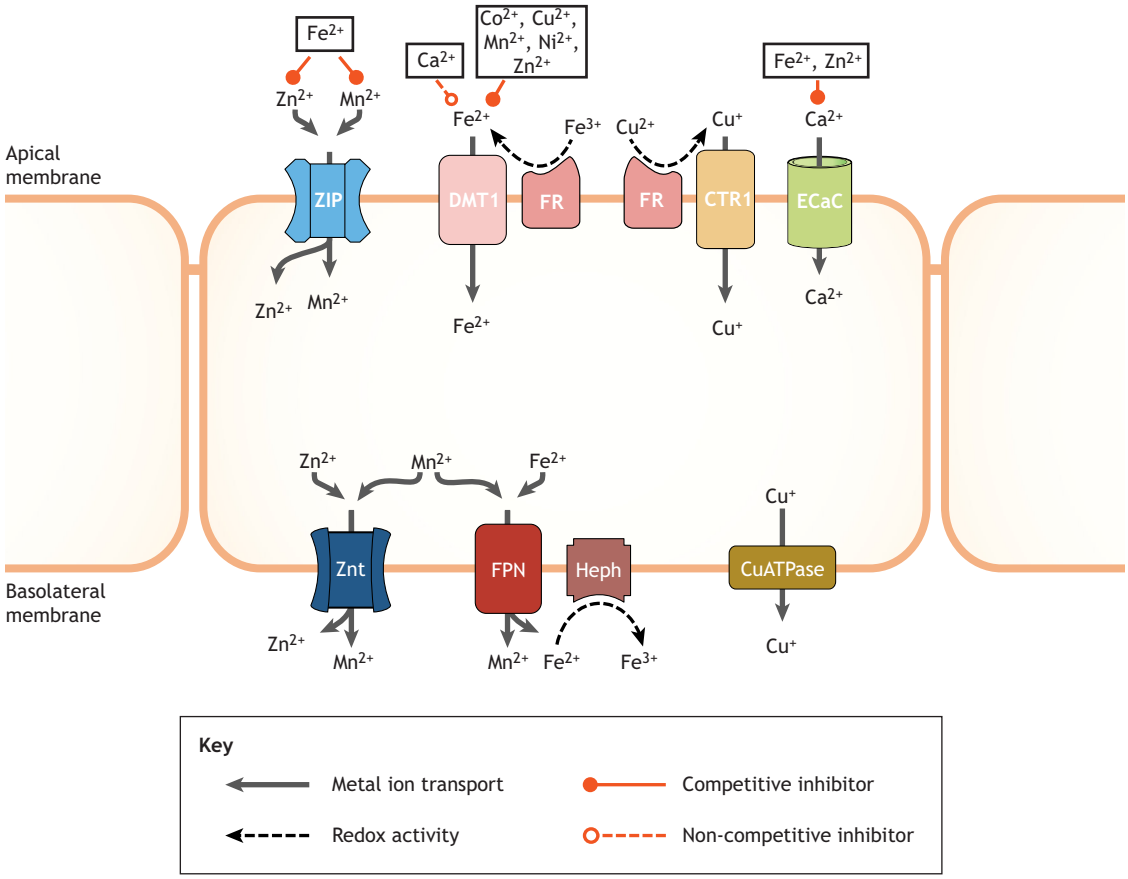

Fig. 1. Proposed model for the transport and interactions among essential trace metals in the transport epithelia of fish. There are complex interactions among trace metals in the process of transepithelial transport. The interactions can be competitive (solid orange lines) or non-competitive (dashed orange line). ZIP, Zrt- and Irt-like proteins (i.e. ZIP8 and ZIP14); DMT1, divalent metal transporter-1; FR, ferric reductase; CTR1, copper transporter-1; ECaC, epithelial calcium channel; ZnT, zinc transporter; FPN, ferroportin; Heph, hephaestin; CuATPase, copper transporting ATPase.

transferrin receptors, can be regulated post-transcriptionally. These proteins have an iron-responsive element (IRE) located either on the 3′- or 5′-untranslated region of the mRNA (Eisenstein, 2000). This region allows for the binding of iron regulatory proteins (IRPs) to the IRE, which can alter mRNA stability and translation of these proteins. IRPs can act as either a translational activator (e.g. binding to the 3′-end of an mRNA to protect against endonuclease cleavage) or a translational inhibitor (e.g. binding to the 5′-end of an mRNA to block the translation process) (Pantopoulos, 2004) (Fig. 2). Another mechanism to regulate the expression of metal storage proteins is the activation of the metal-responsive element (i.e. DNA-binding motif) by the zinc finger metal-responsive transcription factor (i.e. MTF1). Such induction enhances the transcription of the metal-binding protein metallothionein (MT) to store excess zinc and copper (Chen et al., 2020; Tapia et al., 2004). The induction of MT by excess zinc and copper and its role in sequestering free metals are well documented in fish (Bervoets et al., 2013; De Boeck et al., 2003b; Hashemi et al., 2008; McDonald et al., 2021; Wang et al., 2014). However, the mechanism underpinning MTF1–MT activiation by metals is not completely understood. Mulitple models for MTF1–MT activiation have been proposed, including: (i) direct activation of MTF1 by excess zinc in the cytosol, (ii) displacement of zinc from MT by other metals, (iii) stimulation of MTF1 phosphorylation and thereby its transactivation activity, and (iv) promotion of MTF1 translocation to the nucleus through conformational changes and uncovering of the nuclear localization motif (LaRochelle et al., 2001; Smirnova et al., 2000; Wang et al., 2014).

Certain metals can also be regulated by hormones. For example, hepcidin is a peptide hormone that acts as a central regulator of iron homeostasis. It functions by promoting the internalization and degradation of ferroportin at the basolateral membrane during iron overload (Nemeth et al., 2004). In both mammals and fish, hepcidin is primarily synthesized in the liver (Chandrapalan and Kwong, 2020; Nemeth et al., 2004). Several studies have suggested that the mRNA expression of hepcidin is positively regulated by iron in fish (e.g. Neves et al., 2017; Wang and Wang, 2016). Likewise, microinjection of hepcidin cRNA decreases whole-body iron content in zebrafish experiencing iron overload (Jiang et al., 2019). Moreover, intraperitoneal injection of adrenaline or triiodothyronine (T_3) has been found to reduce the activity of ferric reductase in various organs of climbing perch (*Anabas testudineus*), and the effects appeared to be dependent on the feeding status of fish (Rejitha and Peter, 2013). Several previous studies have also shown that trace metal exposure can affect the production of the stress hormone cortisol in fish (Gagnon et al., 2006; Tellis et al., 2012); however, whether cortisol can directly influence metal transport has not been fully characterized. Interestingly, cortisol is reported to reduce copper absorption by common carp (De Boeck et al., 2003a), whereas it stimulates zinc uptake in rainbow trout (Bury et al., 2008). These results suggest that cortisol may have different effects on the uptake of different

Journal of Experimental Biology (2021) 224, jeb238790. doi:10.1242/jeb.238790

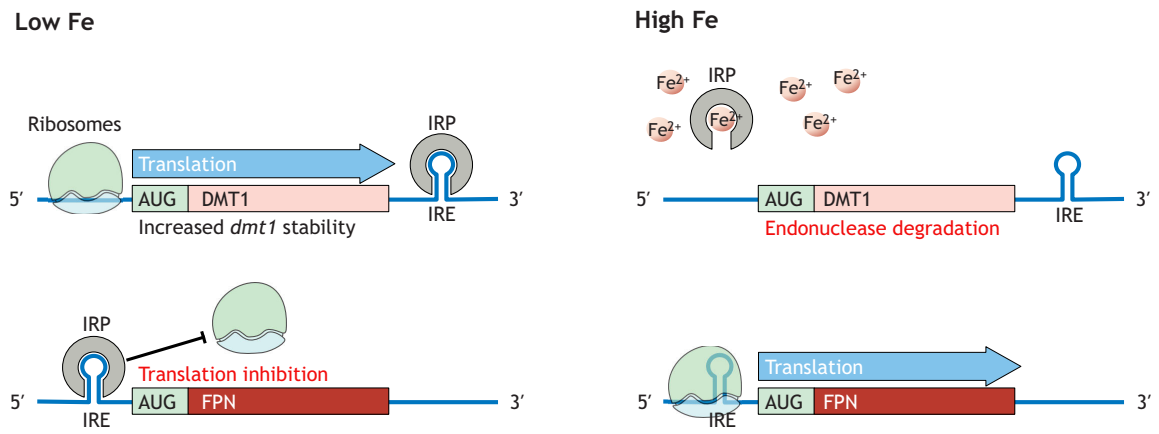

Fig. 2. Cellular sensing and signalling mechanisms in the regulation of the expression of metal transport- and metabolism-related proteins. Several iron transport- and metabolism-related proteins can be regulated post-transcriptionally through the actions between the iron-regulatory protein (IRP) and iron-responsive element (IRE). The IRE is located either in the 3′- or 5′-untranslated regions of the mRNA. This region allows for the binding of IRP to the IRE, which can alter mRNA stability and translation for these proteins. IRP can act as either a translational activator (e.g. binding to the 3′-end of an mRNA to protect against endonuclease cleavage) or a translational inhibitor (e.g. binding to the 5′-end of an mRNA to block the translation process). Alternatively, excess iron may bind to IRP, which prevents IRP from acting on the IRE. Divalent metal transport-1 (DMT1) and ferroportin (FPN) are used as examples in this illustration. AUG denotes the start codon.

metals. Alternatively, cortisol is known to promote major ion uptake by increasing the expression of ion transporters and the density of ion-transporting cells, including those that facilitate the uptake of calcium and sodium (Guh et al., 2015; Kwong et al., 2016a). These pathways may also mediate the uptake of essential trace metals (see above). Additionally, there is emerging evidence from mammalian studies that the status of essential metals in the body may affect various endocrine systems, such as iron in gastrin activity and copper in oxytocin signalling (Stevenson et al., 2019). The mechanism underlying the interplay between essential trace metals and hormonal status in fish is a relatively poorly studied area and requires further characterization.

Abiotic factors influencing metal regulation and homeostasis

The regulation of essential trace metals can be influenced by many environmental/abiotic factors. The effects of water chemistry (e.g. water pH, hardness, dissolved organic matter) on the branchial uptake of trace metals are primarily due to its influence on metal bioavailability and are often discussed in the context of toxicology. The toxicology of trace metals is beyond the scope of the present Review, and we refer the readers to other articles (e.g. Wood et al., 2011). In this section, we provide an overview of our understanding of the influence of temperature, dissolved oxygen content and GIT chemistry/diet composition on the transport and metabolism of essential metals in fish.

The temperature in aquatic environments fluctuates throughout the day according to the day/night cycle and seasonally, and could also be affected by climate change. Many studies have demonstrated that trace metal absorption is positively related to water temperature in fish. For example, zebrafish conditioned to elevated water temperature exhibited an increase in copper accumulation (Pilehvar et al., 2019). Similarly, zinc uptake was found to increase in rainbow trout acclimated to increased water temperature (Glover et al., 2003). In common carp and turbot (*Scophthalmus maximus*), the assimilation efficiency of dietary zinc was shown to increase with increasing water temperature (Pouil et al., 2018; Van Campenhout et al., 2007). One possible reason for the increased metal absorption at higher temperatures could be associated with the general increase in metabolic activities and ventilation rates. For instance, when metabolic parameters were compared in common carp at 10°C and 20°C, standard metabolic rate, maximum metabolic rate and aerobic scope were elevated at the higher temperature (Pillet et al., 2021). In female zebrafish, increases in water temperature induce the hepatic expression of metallothioneins, but not *ctr1* nor *zip8* (Guo et al., 2018b). In contrast, a transient increase in the branchial expression of *ctr1* was observed in common carp during acclimation to increased water temperature (Castaldo et al., 2021). In another study with common carp, however, changes in water temperature were found to have no effect on copper and zinc accumulation in the gill (Pillet et al., 2021). Therefore, it seems likely that changes in water temperature may affect the expression of metal transporters in a tissue-specific manner and that different species may have different capacities to regulate and maintain trace metal balance during acclimation to elevated water temperature. Additionally, the different responses to temperature stress can also be attributed to differences in acclimation duration and the magnitude of temperature changes. Interestingly, cold stress was also found to increase the mRNA abundance of ceruloplasmin, transferrin and metalloreductase (i.e. steap3; reduces both Fe^{3+} and Cu^{2+} ions) in zebrafish larvae (Long et al., 2015). These proteins facilitate metal transport in the circulatory system and endosomal entry of metals into cells; however, the physiological significance of these increases by cold stress requires further study. Finally, the temperature can have indirect effects on the absorption of trace metals from the GIT. For example, an increase in temperature is known to affect GIT physiology by altering phospholipid membrane compositions and intestinal barrier function in fish (Fadhlaoui and Couture, 2016; Sundh et al., 2010).

In mammals, exposure to hypoxia can induce the expression of a variety of iron transport-related proteins through the activation of the hypoxia-inducible factors (HIF) (Renassia and Peyssonnaux, 2019). The increases in iron transport-related proteins (e.g. DMT1, transferrin receptor) by hypoxia are probably due to the increases in iron demand for enhancing oxygen transport capacity (Renassia and Peyssonnaux, 2019). Direct evidence that links the HIF-signalling pathway to micronutrient balance in fish is currently limited to ionic regulation (e.g. calcium) (Kwong et al., 2016b); nevertheless, because HIF signalling is highly conserved among vertebrates, this

signalling pathway is also thought to control the expression of iron transport proteins in fish (Pelster and Egg, 2018). In agreement with this hypothesis, a study with larval zebrafish has shown that hypoxia exposure increases mRNA expression of transferrin, metalloreductase and ceruloplasmin (Long et al., 2015). Genes that are involved in oxygen transport and the heme biosynthesis process are also induced by hypoxic treatment (Long et al., 2015). Interestingly, reduction in dissolved oxygen does not affect iron uptake across the skin and GIT of Pacific hagfish, likely because of their tolerance to hypoxic conditions (Glover et al., 2016).

The transport and metabolism of metals are influenced by the chemistry in the GIT. For example, in rainbow trout, the intestinal absorption of copper was found to decrease when luminal pH was increased from 7.4 to 8.0 (Nadella et al., 2007). Similarly, a reduction in intestinal iron absorption was observed when luminal pH was raised to a more alkaline condition (Kwong et al., 2010). In contrast, the presence of the reducing agent ascorbic acid, which promotes the conversion from ferric iron to ferrous iron, was shown to enhance the intestinal uptake of iron in various fish species including rainbow trout, European flounder and gulf toadfish (*Opsanus beta*) (Bury et al., 2001; Cooper et al., 2006b; Kwong and Niyogi, 2008). These observations are likely a result of the transport properties of DMT1 (a Fe^{2+}/H^{+} symporter), which preferentially transports divalent metals and is driven by a proton gradient (Gunshin et al., 1997). In Atlantic salmon, dietary supplementation with long-chain polyunsaturated fatty acids of the n-3 family [eicosapentaenoic acid/docosahexaenoic acid (EPA/DHA)] has been found to reduce hepatic iron content, which appears to be associated with a decrease in the mRNA abundance of transferrin and ferritin in the liver (Rørvik et al., 2003). In rainbow trout, it has also been suggested that copper–histidine and zinc–histidine complexes can be directly absorbed through specific amino acid transporters (Glover and Wood, 2008; Glover et al., 2003). These results indicated that diet composition has a significant impact on trace metal uptake and metabolism in fish.

Conclusions and perspectives

Iron, copper, zinc and manganese are constituents of an array of metalloproteins and act as cofactors to facilitate various enzymatic reactions. They are also involved in a variety of biological processes, including development, oxygen transport and the immune response. In fish, their uptake may occur through specific metal transporters and via major ion transporters. These trace metals can interact at multiple biological levels from transepithelial transport to intracellular and systemic handling. Their interactions may also influence the function and expression of various metal transport- and metabolism-related proteins. All of this crosstalk may subsequently influence the whole-body homeostasis of trace metals in fish. Recent research efforts have revealed the complex processes involved in the regulation of trace metal homeostasis in fish. However, it is still unclear how the various metal transport pathways in the gill and GIT coordinate to maintain trace metal balance in fish. To date, the molecular machinery in the transport and metabolism of essential trace metals in fish is not completely understood. For example, the functional involvement of specific metal transporters and their cellular/subcellular localization in various organs have not been fully characterized. Additionally, most of our understanding of metal–metal interactions in fish relies primarily on measuring changes in mRNA expression levels following exposure or kinetic analysis through competition study. Direct *in vivo* evidence showing the functional significance of specific metal transporters in metal metabolism and interactions in fish has remained limited. Among the metals discussed, the regulation of manganese homeostasis is much less understood (e.g. uptake mechanism and cellular/systemic handling) and should be addressed in future studies. Recent advances in functional genetics may prove beneficial to enhancing our knowledge of the molecular pathways involved in trace metal homeostasis (Zimmer et al., 2019). This strategy may also have the potential for the discovery of novel compensatory mechanisms in response to trace metal dysregulation. Additionally, most previous studies on examining the influence of abiotic factors on trace metal homeostasis were primarily focused on the toxicology context, thus key knowledge gaps exist in how environmental stressors such as hypoxia and temperature affect the physiological regulation of metals. Moreover, our understanding of essential trace metal homeostasis is mostly limited to a few fish species, including some commercially important species and traditional model organisms such as zebrafish and rainbow trout. Fish in different habitats likely employ different physiological strategies to maintain trace metal balance, and therefore future research should address how the regulation of metal transport function may differ across species.

Acknowledgements

We thank Dr Chun Chih Chen for his contributions to the illustrations. We also thank Stefan Galander and the reviewers for their insightful suggestions.

Competing interests

The authors declare no competing or financial interests.

Funding

Our original research was supported by a Discovery Grant (05984) from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Research Chairs Program to R.W.M.K. This work was also supported by the Ontario Graduate Scholarship (OGS) to T.C.

References

Aliko, V., Qirjo, M., Sula, E., Morina, V. and Faggio, C. (2018). Antioxidant defense system, immune response and erythron profile modulation in gold fish, *Carassius auratus*, after acute manganese treatment. *Fish Shellfish Immunol.* **76**, 101-109. doi:10.1016/j.fsi.2018.02.042

Antony Jesu Prabhu, P., Silva, M. S., Kröeckel, S., Holme, M. H., Ørnsrud, R., Amlund, H., Lock, E. J. and Waagbø, R. (2019). Effect of levels and sources of dietary manganese on growth and mineral composition of post-smolt Atlantic salmon fed low fish meal, plant-based ingredient diets. *Aquaculture* **512**, 734287. doi:10.1016/j.aquaculture.2019.734287

Arredondo, M., Muñoz, P., Mura, C. V. and Núñez, M. T. (2003). DMT1, a physiologically relevant apical Cu^{1+} transporter of intestinal cells. *Am. J. Physiol. Cell Physiol.* **284**, C1525-C1530. doi:10.1152/ajpcell.00480.2002

Baker, Z. N., Cobine, P. A. and Leary, S. C. (2017). The mitochondrion: a central architect of copper homeostasis. *Metallomics* **9**, 1501-1512. doi:10.1039/C7MT00221A

Bakthavatsalam, S., Sharma, S. D., Sonawane, M., Thirumalai, V. and Datta, A. (2014). A zebrafish model of manganism reveals reversible and treatable symptoms that are independent of neurotoxicity. *Dis. Model. Mech.* **7**, 1239-1251. doi:10.1242/dmm.016683

Beaver, L. M., Nkrumah-Elie, Y. M., Truong, L., Barton, C. L., Knecht, A. L., Gonnerman, G. D., Wong, C. P., Tanguay, R. L. and Ho, E. (2017). Adverse effects of parental zinc deficiency on metal homeostasis and embryonic development in a zebrafish model. *J. Nutr. Biochem.* **43**, 78-87. doi:10.1016/j.jnutbio.2017.02.006

Bervoets, L., Knapen, D., De Jonge, M., Van Campenhout, K. and Blust, R. (2013). Differential hepatic metal and metallothionein levels in three feral fish species along a metal pollution gradient. *PLoS ONE* **8**, 60805. doi:10.1371/journal.pone.0060805

Bo, L., Liu, Z., Zhong, Y., Huang, J., Chen, B., Wang, H. and Xu, Y. (2016). Iron deficiency anemia's effect on bone formation in zebrafish mutant. *Biochem. Biophys. Res. Commun.* **475**, 271-276. doi:10.1016/j.bbrc.2016.05.069

Bourassa, D., Gleber, S.-C., Vogt, S., Yi, H., Will, F., Richter, H., Shin, C. H. and Fahrni, C. J. (2014). 3D imaging of transition metals in the zebrafish embryo by X-ray fluorescence microtomography. *Metallomics* **6**, 1648-1655. doi:10.1039/C4MT00121D

Bury, N. (2003). Iron acquisition by teleost fish. *Comp. Biochem. Physiol. C* **135**, 97-105. doi:10.1016/S1532-0456(03)00021-8
Bury, N. R. and Glover, C. N. (2003). Nutritive metal uptake in teleost fish. *J. Exp. Biol.* **206**, 11-23. doi:10.1242/jeb.00068
Bury, N. R., Grosell, M., Wood, C. M., Hogstrand, C., Wilson, R. W., Rankin, J. C., Busk, M., Lecklin, T. and Jensen, F. B. (2001). Intestinal iron uptake in the European flounder (*Platichthys flesus*). *J. Exp. Biol.* **204**, 3779-3787. doi:10.1242/jeb.204.21.3779
Bury, N. R., Chung, M. J., Sturm, A., Walker, P. A. and Hogstrand, C. (2008). Cortisol stimulates the zinc signaling pathway and expression of metallothioneins and ZnT1 in rainbow trout gill epithelial cells. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294**, 623-629. doi:10.1152/ajpregu.00646.2007
Bury, N. R., Boyle, D. and Cooper, C. A. (2011). Iron. In *Fish Physiology* (ed. C. M. Wood, A. P. Farrell and C. J. Brauner), pp. 201-251. Academic Press.
Carriquiriborde, P., Handy, R. D. and Davies, S. (2003). Physiological modulation of iron metabolism in rainbow trout (*Oncorhynchus mykiss*) fed low and high iron diets. *J. Exp. Biol.* **207**, 75-86. doi:10.1242/jeb.00712
Carvalho, L. and Heisenberg, C. P. (2010). The yolk syncytial layer in early zebrafish development. *Trends Cell Biol.* **20**, 586-592. doi:10.1016/j.tcb.2010.06.009
Castaldo, G., Pillet, M., Ameryckx, L., Bervoets, L., Town, R. M., Blust, R. and De Boeck, G. (2021). Temperature effects during a sublethal chronic metal mixture exposure on common carp (*Cyprinus carpio*). *Front. Physiol.* **12**, 1-16. doi:10.3389/fphys.2021.651584
Chanda, S., Paul, B. N., Ghosh, K. and Giri, S. S. (2015). Dietary essentiality of trace minerals in aquaculture-A Review. *Agric. Rev.* **36**, 100-112. doi:10.5958/0976-0741.2015.00012.4
Chandrapalan, T. and Kwong, R. W. M. (2020). Influence of dietary iron exposure on trace metal homeostasis and expression of metal transporters during development in zebrafish. *Environ. Pollut.* **261**, 114159. doi:10.1016/j.envpol.2020.114159
Chen, Q. L., Luo, Z., Wu, K., Huang, C., Zhuo, M. Q., Song, Y. F. and Hu, W. (2015). Differential effects of dietary copper deficiency and excess on lipid metabolism in yellow catfish *Pelteobagrus fulvidraco*. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **184**, 19-28. doi:10.1016/j.cbpb.2015.02.004
Chen, J., Jiang, Y., Shi, H., Peng, Y., Fan, X. and Li, C. (2020). The molecular mechanisms of copper metabolism and its roles in human diseases. *Pflugers Arch. Eur. J. Physiol.* **472**, 1415-1429. doi:10.1007/s00424-020-02412-2
Cheng, J., Luo, Z., Chen, G. H., Wei, C. C. and Zhuo, M. Q. (2017). Identification of eight copper (Cu) uptake related genes from yellow catfish *Pelteobagrus fulvidraco*, and their tissue expression and transcriptional responses to dietborne Cu exposure. *J. Trace Elem. Med. Biol.* **44**, 256-265. doi:10.1016/j.jtemb.2017.09.004
Clearwater, S. J., Farag, A. M. and Meyer, J. S. (2002). Bioavailability and toxicity of dietborne copper and zinc to fish. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **132**, 269-313. doi:10.1016/S1532-0456(02)00078-9
Cooper, C. A., Handy, R. D. and Bury, N. R. (2006a). The effects of dietary iron concentration on gastrointestinal and branchial assimilation of both iron and cadmium in zebrafish (*Danio rerio*). *Aquat. Toxicol.* **79**, 167-175. doi:10.1016/j.aquatox.2006.06.008
Cooper, C. A., Bury, N. R. and Grosell, M. (2006b). The effects of pH and the iron redox state on iron uptake in the intestine of a marine teleost fish, gulf toadfish (*Opsanus beta*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **143**, 292-298. doi:10.1016/j.cbpa.2005.11.024
Cooper, C. A., Shayeghi, M., Techau, M. E., Capdevila, D. M., MacKenzie, S., Durrant, C. and Bury, N. R. (2007). Analysis of the rainbow trout solute carrier 11 family reveals iron import ⩽ pH 7.4 and a functional isoform lacking transmembrane domains 11 and 12. *FEBS Lett.* **581**, 2599-2604. doi:10.1016/j.febslet.2007.04.081
Craig, P. M., Galus, M., Wood, C. M. and McClelland, G. B. (2008). Dietary iron alters waterborne copper-induced gene expression in soft water acclimated zebrafish (*Danio rerio*). *AJP Regul. Integr. Comp. Physiol.* **296**, R362-R373. doi:10.1152/ajpregu.90581.2008
Cuesta, A., Meseguer, J. and Esteban, M. Á. (2008). The antimicrobial peptide hepcidin exerts an important role in the innate immunity against bacteria in the bony fish gilthead seabream. *Mol. Immunol.* **45**, 2333-2342. doi:10.1016/j.molimm.2007.11.007
Dalziel, A. C., Moyes, C. D., Fredriksson, E. and Lougheed, S. C. (2006). Molecular evolution of cytochrome c oxidase in high-performance fish (Teleostei: Scombroidei). *J. Mol. Evol.* **62**, 319-331. doi:10.1007/s00239-005-0110-7
Das, S. and Sahoo, P. K. (2018). Ceruloplasmin, a moonlighting protein in fish. *Fish Shellfish Immunol.* **82**, 460-468. doi:10.1016/j.fsi.2018.08.043
Davis, D. A. and Gatlin, D. M. (1996). Dietary mineral requirements of fish and marine crustaceans. *Rev. Fish. Sci.* **4**, 75-99. doi:10.1080/10641269609388579
De Boeck, G., De Wachter, B., Vlaeminck, A. and Blust, R. (2003a). Effect of cortisol treatment and/or sublethal copper exposure on copper uptake and heat shock protein levels in common carp, *Cyprinus carpio*. *Environ. Toxicol. Chem.* **22**, 1122-1126. doi:10.1002/etc.5620220521
De Boeck, G., Ngo, T. T. H., Van Campenhout, K. and Blust, R. (2003b). Differential metallothionein induction patterns in three freshwater fish during sublethal copper exposure. *Aquat. Toxicol.* **65**, 413-424. doi:10.1016/S0166-445X(03)00178-4
Deforest, D. K. and Meyer, J. S. (2015). Critical review: toxicity of dietborne metals to aquatic organisms. *Crit. Rev. Environ. Sci. Technol.* **45**, 1176-1241. doi:10.1080/10643389.2014.955626
de Souza, P. C. and Bonilla-Rodriguez, G. O. (2007). Fish hemoglobins. *Brazilian J. Med. Biol. Res.* **40**, 769-778. doi:10.1590/S0100-879X2007000600004
Do, J. W., Saravanan, M., Nam, S. E., Lim, H. J. and Rhee, J. S. (2019). Waterborne manganese modulates immunity, biochemical, and antioxidant parameters in the blood of red seabream and black rockfish. *Fish Shellfish Immunol.* **88**, 546-555. doi:10.1016/j.fsi.2019.03.020
Donovan, A., Brownlie, A., Zhou, Y., Shepard, J., Pratt, S. J., Moynihan, J., Paw, B. H., Drejer, A., Barut, B., Zapata, A. et al. (2000). Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* **403**, 776-781. doi:10.1038/35001596
Donovan, A., Brownlie, A., Dorschner, M. O., Zhou, Y., Pratt, S. J., Paw, B. H., Phillips, R. B., Thisse, C., Thisse, B., Zon, L. I. (2002). The zebrafish mutant gene chardonnay (cdy) encodes divalent metal transporter 1 (DMT1). *Blood* **100**, 4655-4659. doi:10.1182/blood-2002-04-1169
Donovan, A., Lima, C. A., Pinkus, J. L., Pinkus, G. S., Zon, L. I., Robine, S. and Andrews, N. C. (2005). The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metab.* **1**, 191-200. doi:10.1016/j.cmet.2005.01.003
Drakesmith, H. and Prentice, A. M. (2012). Hepcidin and the iron-infection axis. *Science (80-.)* **338**, 768-772. doi:10.1126/science.1224577
Dymowska, A. K., Boyle, D., Schultz, A. G. and Goss, G. G. (2015). The role of acid-sensing ion channels in epithelial Na+ uptake in adult zebrafish (*Danio rerio*). *J. Exp. Biol.* **218**, 1244-1251. doi:10.1242/jeb.113118
Eisenstein, R. S. (2000). Iron regulatory proteins and the molecular control of mammalian iron metabolism. *Annu. Rev. Phys. Chem.* **51**, 355-380. doi:10.1146/annurev.physchem.51.1.355
Elvitigala, D. A. S., Premachandra, H. K. A., Whang, I., Oh, M. J., Jung, S. J., Park, C. J. and Lee, J. (2013). A teleostean counterpart of ferritin M subunit from rock bream (*Oplegnathus fasciatus*): an active constituent in iron chelation and DNA protection against oxidative damage, with a modulated expression upon pathogen stress. *Fish Shellfish Immunol.* **35**, 1455-1465. doi:10.1016/j.fsi.2013.08.012
Fadhlaoui, M. and Couture, P. (2016). Combined effects of temperature and metal exposure on the fatty acid composition of cell membranes, antioxidant enzyme activities and lipid peroxidation in yellow perch (*Perca flavescens*). *Aquat. Toxicol.* **180**, 45-55. doi:10.1016/j.aquatox.2016.09.005
Fraenkel, P. G., Traver, D., Donovan, A., Zahrieh, D. and Zon, L. I. (2005). Ferroportin1 is required for normal iron cycling in zebrafish. *J. Clin. Invest.* **115**, 1532-1541. doi:10.1172/JCI23780
Fraenkel, P. G., Gibert, Y., Holzheimer, J. L., Lattanzi, V. J., Burnett, S. F., Dooley, K. A., Wingert, R. A. and Zon, L. I. (2009). Transferrin-a modulates hepcidin expression in zebrafish embryos. *Blood* **113**, 2843-2850. doi:10.1182/blood-2008-06-165340
Gagnon, A., Jumarie, C. and Hontela, A. (2006). Effects of Cu on plasma cortisol and cortisol secretion by adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* **78**, 59-65. doi:10.1016/j.aquatox.2006.02.004
Gallaugher, P., Thorarensen, H. and Farrell, A. P. (1995). Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). *Respir. Physiol.* **102**, 279-292. doi:10.1016/0034-5687(95)00065-8
Gansner, J. M., Mendelsohn, B. A., Hultman, K. A., Johnson, S. L. and Gitlin, J. D. (2007). Essential role of lysyl oxidases in notochord development. *Dev. Biol.* **307**, 202-213. doi:10.1016/j.ydbio.2007.04.029
Ganz, T. (2013). Systemic iron homeostasis. *Physiol. Rev.* **93**, 1721-1741. doi:10.1152/physrev.00008.2013
Garrick, M. D., Dolan, K. G., Horbinski, C., Ghio, A. J., Higgins, D., Porubcin, M., Moore, E. G., Hainsworth, L. N., Umbreit, J. N., Conrad, M. E. et al. (2003). DMT1: A mammalian transporter for multiple metals. *Biometals* **16**, 41-54. doi:10.1023/A:1020702213099
Glover, C. N. and Wood, C. M. (2008). Absorption of copper and copper-histidine complexes across the apical surface of freshwater rainbow trout intestine. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **178**, 101-109. doi:10.1007/s00360-007-0203-2
Glover, C. N., Bury, N. R. and Hogstrand, C. (2003). Zinc uptake across the apical membrane of freshwater rainbow trout intestine is mediated by high affinity, low affinity, and histidine-facilitated pathways. *Biochim. Biophys. Acta Biomembr.* **1614**, 211-219.
Glover, C. N., Niyogi, S., Blewett, T. A. and Wood, C. M. (2016). Iron transport across the skin and gut epithelia of Pacific hagfish: kinetic characterisation and effect of hypoxia. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **199**, 1-7. doi:10.1016/j.cbpa.2016.04.018
Grillo, A. S., SantaMaria, A. M., Kafina, M. D., Cioffi, A. G., Huston, N. C., Han, M., Seo, Y. A., Yien, Y. Y., Nardone, C., Menon, A. V. et al. (2017). Restored iron transport by a small molecule promotes absorption and hemoglobinization in animals. *Science (80-.)* **356**, 608-616. doi:10.1126/science.aah3862

Grosell, M. and Wood, C. M. (2002). Copper uptake across rainbow trout gills: mechanisms of apical entry. *J. Exp. Biol.* **205**, 1179-1188. doi:10.1242/jeb.205.8.1179

Guh, Y.-J., Lin, C.-H. and Hwang, P.-P. (2015). Osmoregulation in zebrafish: ion transport mechanisms and functional regulation. *EXCLI J.* **14**, 627-659.

Gunshin, H., Mackenzie, B., Berger, U. V., Gunshin, Y., Romero, M. F., Boron, W. F., Nussberger, S., Gollan, J. L. and Hediger, M. A. (1997). Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* **388**, 482-488. doi:10.1038/41343

Guo, Z., Zhang, W., Du, S., Green, I., Tan, Q. and Zhang, L. (2016). Developmental patterns of copper bioaccumulation in a marine fish model *Oryzias melastigma*. *Aquat. Toxicol.* **170**, 216-222. doi:10.1016/j.aquatox.2015.11.026

Guo, Y. L., Wu, P., Jiang, W. D., Liu, Y., Kuang, S. Y., Jiang, J., Tang, L., Tang, W. N., Zhang, Y. A., Zhou, X. Q. et al. (2018a). The impaired immune function and structural integrity by dietary iron deficiency or excess in gill of fish after infection with *Flavobacterium columnare*: regulation of NF-κB, TOR, JNK, p38MAPK, Nrf2 and MLCK signalling. *Fish Shellfish Immunol.* **74**, 593-608. doi:10.1016/j.fsi.2018.01.027

Guo, S. N., Zheng, J. L., Yuan, S. S. and Zhu, Q. L. (2018b). Effects of heat and cadmium exposure on stress-related responses in the liver of female zebrafish: Heat increases cadmium toxicity. *Sci. Total Environ.* **618**, 1363-1370. doi:10.1016/j.scitotenv.2017.09.264

Haller, G., McCall, K., Jenkitkasemwong, S., Sadler, B., Antunes, L., Nikolov, M., Whittle, J., Upshaw, Z., Shin, J., Baschal, E. et al. (2018). A missense variant in SLC39A8 is associated with severe idiopathic scoliosis. *Nat. Commun.* **9**, 4171. doi:10.1038/s41467-018-06705-0

Handy, R. D., Musonda, M. M., Phillips, C. and Falla, S. J. (2000). Mechanisms of gastrointestinal copper absorption in the African walking catfish: copper dose-effects and a novel anion-dependent pathway in the intestine. *J. Exp. Biol.* **203**, 2365-2377. doi:10.1242/jeb.203.15.2365

Handy, R. D., Eddy, F. B. and Baines, H. (2002). Sodium-dependent copper uptake across epithelia: a review of rationale with experimental evidence from gill and intestine. *Biochim. Biophys. Acta Biomembr* **1566**, 104-115.

Harris, E. D. (1992). Copper as a cofactor and regulator of copper,zinc superoxide dismutase. *J. Nutr.* **122**, 636-640. doi:10.1093/jn/122.suppl_3.636

Hashemi, S., Kunwar, P. S., Blust, R. and De Boeck, G. (2008). Differential metallothionein induction patterns in fed and starved carp (*Cyprinus carpio*) during waterborne copper exposure. *Environ. Toxicol. Chem.* **27**, 2154-2158. doi:10.1897/07-502.1

Hassan, A. T. and Kwong, R. W. M. (2020). The neurophysiological effects of iron in early life stages of zebrafish. *Environ. Pollut.* **267**, 115625. doi:10.1016/j.envpol.2020.115625

Hellman, N. E. and Gitlin, J. D. (2002). Ceruloplasmin metabolism and function. *Annu. Rev. Nutr.* **22**, 439-458. doi:10.1146/annurev.nutr.22.012502.114457

Illing, A. C., Shawki, A., Cunningham, C. L. and Mackenzie, B. (2012). Substrate profile and metal-ion selectivity of human divalent metal-ion transporter-1. *J. Biol. Chem.* **287**, 30485-30496. doi:10.1074/jbc.M112.364208

Ishizaki, H., Spitzer, M., Wildenhain, J., Anastasaki, C., Zeng, Z., Dolma, S., Shaw, M., Madsen, E., Gitlin, J., Marais, R. et al. (2010). Combined zebrafish-yeast chemical-genetic screens reveal gene–copper-nutrition interactions that modulate melanocyte pigmentation. *Dis. Model. Mech.* **3**, 639-651. doi:10.1242/dmm.005769

Iyengar, V., Pullakhandam, R. and Nair, K. M. (2009). Iron-zinc interaction during uptake in human intestinal Caco-2 cell line: kinetic analyses and possible mechanism. *Indian J. Biochem. Biophys.* **46**, 299-306.

Jiang, X. F., Liu, Z. F., Lin, A. F., Xiang, L. X. and Shao, J. Z. (2017). Coordination of bactericidal and iron regulatory functions of hepcidin in innate antimicrobial immunity in a zebrafish model. *Sci. Rep.* **7**, 4265. doi:10.1038/s41598-017-04069-x

Jiang, Y., Chen, B., Yan, Y. and Zhu, G. X. (2019). Hepcidin protects against iron overload-induced inhibition of bone formation in zebrafish. *Fish Physiol. Biochem.* **45**, 365-374. doi:10.1007/s10695-018-0568-z

Kamińska-Gibas, T., Szczygieł, J., Jurecka, P. and Irnazarow, I. (2020). The many faces of transferrin: does genotype modulate immune response? *Fish Shellfish Immunol.* **102**, 511-518. doi:10.1016/j.fsi.2020.05.001

Kaplan, J. H. and Maryon, E. B. (2016). How mammalian cells acquire copper: an essential but potentially toxic metal. *Biophys. J.* **110**, 7-13. doi:10.1016/j.bpj.2015.11.025

Krasnići, N., Dragun, Z., Erk, M. and Raspor, B. (2013). Distribution of selected essential (Co, Cu, Fe, Mn, Mo, Se, and Zn) and nonessential (Cd, Pb) trace elements among protein fractions from hepatic cytosol of European chub (*Squalius cephalus* L.). *Environ. Sci. Pollut. Res.* **20**, 2340-2351. doi:10.1007/s11356-012-1105-8

Kumar, N., Krishnani, K. K., Kumar, P., Jha, A. K., Gupta, S. K. and Singh, N. P. (2017). Dietary zinc promotes immuno-biochemical plasticity and protects fish against multiple stresses. *Fish Shellfish Immunol.* **62**, 184-194. doi:10.1016/j.fsi.2017.01.017

Kwok, M. L. and Chan, K. M. (2019). Functional characterization of copper transporters zCtr1, zAtox1, zAtp7a and zAtp7b in zebrafish liver cell line ZFL. *Metallomics* **11**, 1532-1546. doi:10.1039/C9MT00159J

Kwong, R. W. M. and Niyogi, S. (2008). An *in vitro* examination of intestinal iron absorption in a freshwater teleost, rainbow trout (*Oncorhynchus mykiss*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **178**, 963-975. doi:10.1007/s00360-008-0279-3

Kwong, R. W. M. and Niyogi, S. (2009). The interactions of iron with other divalent metals in the intestinal tract of a freshwater teleost, rainbow trout (Oncorhynchus mykiss). *Comp. Biochem. Physiol. C* **150**, 442-449.

Kwong, R. W. M., Andrés, J. A. J. A. and Niyogi, S. (2010). Molecular evidence and physiological characterization of iron absorption in isolated enterocytes of rainbow trout (Oncorhynchus mykiss): Implications for dietary cadmium and lead absorption. *Aquat. Toxicol.* **99**, 343-350. doi:10.1016/j.aquatox.2010.05.012

Kwong, R. W. M., Hamilton, C. D. C. D. and Niyogi, S. (2013). Effects of elevated dietary iron on the gastrointestinal expression of Nramp genes and iron homeostasis in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* **39**, 363-372. doi:10.1007/s10695-012-9705-2

Kwong, R. W. M., Auprix, D. and Perry, S. F. (2014). Involvement of the calcium-sensing receptor in calcium homeostasis in larval zebrafish exposed to low environmental calcium. *Am. J. Physiol. Integr. Comp. Physiol.* **306**, R211-R221. doi:10.1152/ajpregu.00350.2013

Kwong, R. W. M., Kumai, Y. and Perry, S. F. (2016a). Neuroendocrine control of ionic balance in zebrafish. *Gen. Comp. Endocrinol.* **234**, 40-46. doi:10.1016/j.ygcen.2016.05.016

Kwong, R. W. M., Kumai, Y., Tzaneva, V., Azzi, E., Hochhold, N., Robertson, C., Pelster, B. and Perry, S. F. (2016b). Inhibition of calcium uptake during hypoxia in developing zebrafish is mediated by hypoxia-inducible factor. *J. Exp. Biol.* **219**, 3988-3995. doi:10.1242/jeb.148700

LaRochelle, O., Gagné, V., Charron, J., Soh, J. W. and Séguin, C. (2001). Phosphorylation is involved in the activation of metal-regulatory transcription factor I in response to metal ions. *J. Biol. Chem.* **276**, 41879-41888. doi:10.1074/jbc.M108313200

Leung, K. P., Chen, D. and Chan, K. M. (2014). Understanding copper sensitivity in zebrafish (*Danio rerio*) through the intracellular localization of copper transporters in a hepatocyte cell-line ZFL and the tissue expression profiles of copper transporters. *Metallomics* **6**, 1057-1067. doi:10.1039/C3MT00366C

Li, L. and Yang, X. (2018). The essential element manganese, oxidative stress, and metabolic diseases: links and interactions. *Oxid. Med. Cell. Longev.* **2018**, 7580707.

Lin, C. H. and Hwang, P. P. (2016). The control of calcium metabolism in zebrafish (*Danio rerio*). *Int. J. Mol. Sci.* **17**, 1783. doi:10.3390/ijms17111783

Liu, H., Peatman, E., Wang, W., Abernathy, J., Liu, S., Kucuktas, H., Terhune, J., Xu, D. H., Klesius, P. and Liu, Z. (2011). Molecular responses of ceruloplasmin to *Edwardsiella ictaluri* infection and iron overload in channel catfish (*Ictalurus punctatus*). *Fish Shellfish Immunol.* **30**, 992-997. doi:10.1016/j.fsi.2010.12.033

Long, Y., Yan, J., Song, G., Li, X., Li, X., Li, Q. and Cui, Z. (2015). Transcriptional events co-regulated by hypoxia and cold stresses in Zebrafish larvae. *BMC Genomics* **16**, 385. doi:10.1186/s12864-015-1560-y

Mackenzie, N. C., Brito, M., Reyes, A. E. and Allende, M. L. (2004). Cloning, expression pattern and essentiality of the high-affinity copper transporter 1 (ctr1) gene in zebrafish. *Gene* **328**, 113-120. doi:10.1016/j.gene.2003.11.019

Madejczyk, M. S. and Ballatori, N. (2017). The iron transporter ferroportin can also function as a manganese exporter. *Biochim. Biophys. Acta* **1818**, 651-657. doi:10.1016/j.bbamem.2011.12.002

Makwinja, R. and Geremew, A. (2020). Roles and requirements of trace elements in tilapia nutrition: review. *Egypt. J. Aquat. Res.* **46**, 281-287. doi:10.1016/j.ejar.2020.05.001

Martínez, D., Oyarzún, R., Vargas-Lagos, C., Pontigo, J. P., Soto-Dávila, M., Saravia, J., Romero, A., Núñez, J. J., Yáñez, A. J. and Vargas-Chacoff, L. (2017). Identification, characterization and modulation of ferritin-H in the sub-Antarctic notothenioid *Eleginops maclovinus* challenged with *Piscirickettsia salmonis*. *Dev. Comp. Immunol.* **73**, 88-96. doi:10.1016/j.dci.2017.03.015

Mathews, T. and Fisher, N. S. (2009). Dominance of dietary intake of metals in marine elasmobranch and teleost fish. *Sci. Total Environ.* **407**, 5156-5161. doi:10.1016/j.scitotenv.2009.06.003

McCann, S., Amadó, M. P. and Moore, S. E. (2020). The role of iron in brain development: a systematic review. *Nutrients* **12**, 2001. doi:10.3390/nu12072001

McDonald, S., Hassell, K. and Cresswell, T. (2021). Effect of short-term dietary exposure on metal assimilation and metallothionein induction in the estuarine fish *Pseudogobius* sp. *Sci. Total Environ.* **772**, 145042. doi:10.1016/j.scitotenv.2021.145042

Mebane, C. A., Chowdhury, M. J., De Schamphelaere, K. A. C., Lofts, S., Paquin, P. R., Santore, R. C. and Wood, C. M. (2020). Metal bioavailability models: current status, lessons learned, considerations for regulatory use, and the path forward. *Environ. Toxic. Chem.* **39**, 60-84. doi:10.1002/etc.4560

Mendelsohn, B. A., Yin, C., Johnson, S. L., Wilm, T. P., Solnica-Krezel, L. and Gitlin, J. D. (2006). Atp7a determines a hierarchy of copper metabolism essential for notochord development. *Cell Metab.* **4**, 155-162. doi:10.1016/j.cmet.2006.05.001

Mi, X., Li, Z., Yan, J., Li, Y., Zheng, J., Zhaung, Z., Yang, W., Gong, L. and Shi, J. (2020). Activation of HIF-1 signaling ameliorates liver steatosis in zebrafish atp7b deficiency (Wilson's disease) models. *Biochim. Biophys. Acta Mol. Basis Dis.* **1866**, 165842. doi:10.1016/j.bbadis.2020.165842

Moazenzadeh, K., Rajabi Islami, H., Zamini, A. and Soltani, M. (2020). Effect of dietary inorganic copper on growth performance and some hematological indices of Siberian sturgeon *Acipenser baerii* juveniles. *N. Am. J. Aquac.* **82**, 200-207. doi:10.1002/naaq.10145

Muraina, I. A., Maret, W., Bury, N. R. and Hogstrand, C. (2020). Hatching gland development and hatching in zebrafish embryos: a role for zinc and its transporters Zip10 and Znt1a. *Biochem. Biophys. Res. Commun.* **528**, 698-705. doi:10.1016/j.bbrc.2020.05.131

Nadella, S. R., Grosell, M. and Wood, C. M. (2007). Mechanisms of dietary Cu uptake in freshwater rainbow trout: evidence for Na-assisted Cu transport and a specific metal carrier in the intestine. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **177**, 433-446. doi:10.1007/s00360-006-0142-3

Nadella, S. R., Hung, C. C. Y. and Wood, C. M. (2011). Mechanistic characterization of gastric copper transport in rainbow trout. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **181**, 27-41. doi:10.1007/s00360-010-0510-x

Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. D., Donovan, A., Ward, D. M. V., Ganz, T. and Kaplan, J. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science (80-.).* **306**, 2090-2093. doi:10.1126/science.1104742

Neves, J. V., Wilson, J. M. and Rodrigues, P. N. S. (2009). Transferrin and ferritin response to bacterial infection: The role of the liver and brain in fish. *Dev. Comp. Immunol.* **33**, 848-857. doi:10.1016/j.dci.2009.02.001

Neves, J. V., Ramos, M. F., Moreira, A. C., Silva, T., Gomes, M. S. and Rodrigues, P. N. S. (2017). Hamp1 but not Hamp2 regulates ferroportin in fish with two functionally distinct hepcidin types. *Sci. Rep.* **7**, 14793. doi:10.1038/s41598-017-14933-5

Niyogi, S. and Wood, C. M. (2006). Interaction between dietary calcium supplementation and chronic waterborne zinc exposure in juvenile rainbow trout, *Oncorhynchus mykiss*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **143**, 94-102. doi:10.1016/j.cbpc.2005.12.007

Ong, S. T., Shan Ho, J. Z., Ho, B. and Ding, J. L. (2006). Iron-withholding strategy in innate immunity. *Immunobiology* **211**, 295-314. doi:10.1016/j.imbio.2006.02.004

Pan, T.-C., Liao, B.-K., Huang, C.-J., Lin, L.-Y. and Hwang, P.-P. (2005). Epithelial Ca^{2+} channel expression and Ca^{2+} uptake in developing zebrafish. *Am. J. Physiol. Integr. Comp. Physiol.* **289**, R1202-R1211. doi:10.1152/ajpregu.00816.2004

Pantopoulos, K. (2004). Iron metabolism and the IRE/IRP regulatory system: an update. *Ann. N. Y. Acad. Sci.* **1012**, 1-13. doi:10.1196/annals.1306.001

Pelster, B. and Egg, M. (2018). Hypoxia-inducible transcription factors in fish: expression, function and interconnection with the circadian clock. *J. Exp. Biol.* **221**, 163709. doi:10.1242/jeb.163709

Pilehvar, A., Town, R. M. and Blust, R. (2019). The effect of thermal pre-incubation and exposure on sensitivity of zebrafish (*Danio rerio*) to copper and cadmium single and binary exposures. *Aquat. Toxicol.* **213**, 105226. doi:10.1016/j.aquatox.2019.105226

Pillet, M., Castaldo, G., Rodgers, E. M., Poleksić, V., Rašković, B., Bervoets, L., Blust, R. and De Boeck, G. (2021). Physiological performance of common carp (*Cyprinus carpio*, L., 1758) exposed to a sublethal copper/zinc/cadmium mixture. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **242**, 108954. doi:10.1016/j.cbpc.2020.108954

Pouil, S., Oberhänsli, F., Bustamante, P. and Metian, M. (2018). Investigations of temperature and pH variations on metal trophic transfer in turbot (*Scophthalmus maximus*). *Environ. Sci. Pollut. Res.* **25**, 11219-11225. doi:10.1007/s11356-017-8691-4

Prasad, A. S. (2008). Zinc in human health: effect of zinc on immune cells. *Mol. Med.* **14**, 353. doi:10.2119/2008-00033.Prasad

Puar, P., Niyogi, S. and Kwong, R. W. M. (2020). Regulation of metal homeostasis and zinc transporters in early-life stage zebrafish following sublethal waterborne zinc exposure. *Aquat. Toxicol.* **225**, 105524. doi:10.1016/j.aquatox.2020.105524

Puig, S., Ramos-Alonso, L., Romero, A. M. and Martínez-Pastor, M. T. (2017). The elemental role of iron in DNA synthesis and repair. *Metallomics* **9**, 1483-1500. doi:10.1039/C7MT00116A

Pyle, G. G., Kamunde, C. N., McDonald, D. G. and Wood, C. M. (2003). Dietary sodium inhibits aqueous copper uptake in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **206**, 609-618. doi:10.1242/jeb.00114

Qiu, A. and Hogstrand, C. (2004). Functional characterisation and genomic analysis of an epithelial calcium channel (ECaC) from pufferfish, *Fugu rubripes*. *Gene* **342**, 113-123. doi:10.1016/j.gene.2004.07.041

Rae, T. D., Schmidt, P. J., Pufahl, R. A., Culotta, V. C. and O'Halloran, T. V. (1999). Undetectable intracellular free copper: The requirement of a copper chaperone for superoxide dismutase. *Science* **284**, 805-808. doi:10.1126/science.284.5415.805

Rejitha, V. and Peter, M. C. S. (2013). Adrenaline and triiodothyronine modify the iron handling in the freshwater air-breathing fish *Anabas testudineus* Bloch: role of ferric reductase in iron acquisition. *Gen. Comp. Endocrinol.* **181**, 130-138. doi:10.1016/j.ygcen.2012.11.008

Renassia, C. and Peyssonnaux, C. (2019). New insights into the links between hypoxia and iron homeostasis. *Curr. Opin. Hematol.* **26**, 125-130. doi:10.1097/MOH.0000000000000494

Riggio, M., Filosa, S., Parisi, E. and Scudiero, R. (2003). Changes in zinc, copper and metallothionein contents during oocyte growth and early development of the teleost *Danio rerio* (zebrafish). *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **135**, 191-196. doi:10.1016/S1532-0456(03)00107-8

Rørvik, K. A., Dehli, A., Thomassen, M., Ruyter, B., Steien, S. H. and Salte, R. (2003). Synergistic effects of dietary iron and omega-3 fatty acid levels on survival of farmed Atlantic salmon, *Salmo salar* L., during natural outbreaks of furunculosis and cold water vibriosis. *J. Fish Dis.* **26**, 477-485. doi:10.1046/j.1365-2761.2003.00482.x

Sahoo, P. K., Das, S., Mahapatra, K. D., Saha, J. N., Baranski, M., Ødegård, J. and Robinson, N. (2013). Characterization of the ceruloplasmin gene and its potential role as an indirect marker for selection to *Aeromonas hydrophila* resistance in rohu, *Labeo rohita*. *Fish Shellfish Immunol.* **34**, 1325-1334. doi:10.1016/j.fsi.2013.02.020

Scudiero, R., Trinchella, F., Riggio, M. and Parisi, E. (2007). Structure and expression of genes involved in transport and storage of iron in red-blooded and hemoglobin-less Antarctic notothenioids. *Gene* **397**, 1-11. doi:10.1016/j.gene.2007.03.003

Seo, Y. A. and Wessling-Resnick, M. (2015). Ferroportin deficiency impairs manganese metabolism in flatiron mice. *FASEB J.* **29**, 2726-2733. doi:10.1096/fj.14-262592

Shaw, G. C., Cope, J. J., Li, L., Corson, K., Hersey, C., Ackermann, G. E., Gwynn, B., Lambert, A. J., Wingert, R. A., Traver, D. et al. (2006). Mitoferrin is essential for erythroid iron assimilation. *Nature* **440**, 96-100. doi:10.1038/nature04512

Shawki, A. and Mackenzie, B. (2010). Interaction of calcium with the human divalent metal-ion transporter-1. *Biochem. Biophys. Res. Commun.* **393**, 471-475. doi:10.1016/j.bbrc.2010.02.025

Shawki, A., Anthony, S. R., Nose, Y., Engevik, M. A., Niespodzany, E. J., Barrientos, T., Öhrvik, H., Worrell, R. T., Thiele, D. J. and Mackenzie, B. (2015). Intestinal DMT1 is critical for iron absorption in the mouse but is not required for the absorption of copper or manganese. *Am. J. Physiol. Gastrointest. Liver Physiol.* **309**, G635-G647. doi:10.1152/ajpgi.00160.2015

Shi, J. and Camus, A. C. (2006). Hepcidins in amphibians and fishes: antimicrobial peptides or iron-regulatory hormones? *Dev. Comp. Immunol.* **30**, 746-755. doi:10.1016/j.dci.2005.10.009

Shike, H., Lauth, X., Westerman, M. E., Ostland, V. E., Carlberg, J. M., Van Olst, J. C., Shimizu, C., Bulet, P. and Burns, J. C. (2002). Bass hepcidin is a novel antimicrobial peptide induced by bacterial challenge. *Eur. J. Biochem.* **269**, 2232-2237. doi:10.1046/j.1432-1033.2002.02881.x

Smirnova, I. V., Bittel, D. C., Ravindra, R., Jiang, H. and Andrews, G. K. (2000). Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1. *J. Biol. Chem.* **275**, 9377-9384. doi:10.1074/jbc.275.13.9377

Soma, S., Latimer, A. J., Chun, H., Vicary, A. C., Timbalia, S. A., Boulet, A., Rahn, J. J., Chan, S. S. L., Leary, S. C., Kim, B. E. et al. (2018). Elesclomol restores mitochondrial function in genetic models of copper deficiency. *Proc. Natl. Acad. Sci. USA* **115**, 8161-8166. doi:10.1073/pnas.1806296115

Song, Z. X., Jiang, W. D., Liu, Y., Wu, P., Jiang, J., Zhou, X. Q., Kuang, S. Y., Tang, L., Tang, W. N., Zhang, Y. A. et al. (2017). Dietary zinc deficiency reduced growth performance, intestinal immune and physical barrier functions related to NF-κB, TOR, Nrf2, JNK and MLCK signaling pathway of young grass carp (*Ctenopharyngodon idella*). *Fish Shellfish Immunol.* **66**, 497-523. doi:10.1016/j.fsi.2017.05.048

Stevenson, M. J., Uyeda, K. S., Harder, N. H. O. and Heffern, M. C. (2019). Metal-dependent hormone function: The emerging interdisciplinary field of metalloendocrinology. *Metallomics* **11**, 85-110. doi:10.1039/C8MT00221E

Sundh, H., Kvamme, B. O., Fridell, F., Olsen, R. E., Ellis, T., Taranger, G. L. and Sundell, K. (2010). Intestinal barrier function of Atlantic salmon (*Salmo salar* L.) post smolts is reduced by common sea cage environments and suggested as a possible physiological welfare indicator. *BMC Physiol.* **10**, 1-13. doi:10.1186/1472-6793-10-22

Tapia, L., González-Agüero, M., Cisternas, M. F., Suazo, M., Cambiazo, V., Uauy, R. and González, M. (2004). Metallothionein is crucial for safe intracellular copper storage and cell survival at normal and supra-physiological exposure levels. *Biochem. J.* **378**, 617-624. doi:10.1042/bj20031174

Tarifeño-Saldivia, E., Aguilar, A., Contreras, D., Mercado, L., Morales-Lange, B., Márquez, K., Henríquez, A., Riquelme-Vidal, C. and Boltana, S. (2018). Iron overload is associated with oxidative stress and nutritional immunity during viral infection in fish. *Front. Immunol.* **9**, 1296. doi:10.3389/fimmu.2018.01296

Tellis, M. S., Alsop, D. and Wood, C. M. (2012). Effects of copper on the acute cortisol response and associated physiology in rainbow trout. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **155**, 281-289. doi:10.1016/j.cbpc.2011.09.008

Tennant, J., Stansfield, M., Yamaji, S., Srai, S. and Sharp, P. (2002). Effects of copper on the expression of metal transporters in human intestinal Caco-2 cells. *FEBS Lett.* **527**, 239-244. doi:10.1016/S0014-5793(02)03253-2

Thompson, B. A. V., Sharp, P. A., Elliott, R. and Fairweather-Tait, S. J. (2010). Inhibitory effect of calcium on non-heme iron absorption may be related to translocation of DMT-1 at the apical membrane of enterocytes. *J. Agric. Food Chem.* **58**, 8414-8417. doi:10.1021/jf101388z

Thomason, R. T., Pettiglio, M. A., Herrera, C., Kao, C., Gitlin, J. D. and Bartnikas, T. B. (2017). Characterization of trace metal content in the developing zebrafish embryo. *PLoS One* **12**, e0179318. doi:10.1371/journal.pone.0179318

Tuschl, K., Meyer, E., Valdivia, L. E., Zhao, N., Dadswell, C., Abdul-Sada, A., Hung, C. Y., Simpson, M. A., Chong, W. K., Jacques, T. S. et al. (2016). Mutations in SLC39A14 disrupt manganese homeostasis and cause childhood-onset parkinsonism-dystonia. *Nat. Commun.* **7**, 11601. doi:10.1038/ncomms11601

Van Campenhout, K., Bervoets, L. and Blust, R. (2007). Assimilation efficiencies of Cd and Zn in the common carp (*Cyprinus carpio*): effects of metal concentration, temperature and prey type. *Environ. Pollut.* **145**, 905-914. doi:10.1016/j.envpol.2006.05.002

Viegas, M. N., Salgado, M. A., Aguiar, C., Almeida, A., Gavaia, P. and Dias, J. (2021). Effect of dietary manganese and zinc levels on growth and bone status of Senegalese sole (*Solea senegalensis*) post-larvae. *Biol. Trace Elem. Res.* **199**, 2012-2021. doi:10.1007/s12011-020-02307-4

Wang, J.-J. and Sun, L. (2015). Ferritin M of *Paralichthys olivaceus* possesses antimicrobial and antioxidative properties. *Fish Physiol. Biochem.* **41**, 951-959. doi:10.1007/s10695-015-0060-y

Wang, J. and Wang, W.-X. (2016). Novel insights into iron regulation and requirement in marine medaka *Oryzias melastigma*. *Sci. Rep.* **6**, 26615. doi:10.1038/srep26615

Wang, W.-C., Mao, H., Ma, D.-D. and Yang, W.-X. (2014). Characteristics, functions, and applications of metallothionein in aquatic vertebrates. *Front. Mar. Sci.* **1**, 34. doi:10.3389/fmars.2014.00034

Wang, Y., Wu, Y., Li, T., Wang, X. and Zhu, C. (2019). Iron metabolism and brain development in premature infants. *Front. Physiol.* **10**, 463. doi:10.3389/fphys.2019.00463

Watanabe, T., Kiron, V. and Satoh, S. (1997). Trace minerals in fish nutrition. *Aquaculture* **151**, 185-207. doi:10.1016/S0044-8486(96)01503-7

Wessels, I., Maywald, M. and Rink, L. (2017). Zinc as a gatekeeper of immune function. *Nutrients* **9**, 1286. doi:10.3390/nu9121286

Wichmann, L. and Althaus, M. (2020). Evolution of epithelial sodium channels: current concepts and hypotheses. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **319**, R387-R400. doi:10.1152/ajpregu.00144.2020

Wood, C. M., Farrell, A. P. and Brauner, C. J. (2011). *Homeostasis and Toxicology of Essential Metals*. Academic Press.

Wyman, S., Simpson, R. J., McKie, A. T. and Sharp, P. A. (2008). Dcytb (Cybrd1) functions as both a ferric and a cupric reductase *in vitro*. *FEBS Lett.* **582**, 1901-1906. doi:10.1016/j.febslet.2008.05.010

Xia, Z., Wei, J., Li, Y., Wang, J., Li, W., Wang, K., Hong, X., Zhao, L., Chen, C., Min, J. et al. (2017). Zebrafish slc30a10 deficiency revealed a novel compensatory mechanism of Atp2c1 in maintaining manganese homeostasis. *PLoS Genet.* **13**, 1-29.

Xue, Y., Gao, S. and Liu, F. (2015). Genome-wide analysis of the zebrafish Klf family identifies two genes important for erythroid maturation. *Dev. Biol.* **403**, 115-127. doi:10.1016/j.ydbio.2015.05.015

Yamaji, S., Tennant, J., Tandy, S., Williams, M., Singh Srai, S. K. and Sharp, P. (2001). Zinc regulates the function and expression of the iron transporters DMT1 and IREG1 in human intestinal Caco-2 cells. *FEBS Lett.* **507**, 137-141. doi:10.1016/S0014-5793(01)02953-2

Yan, G., Zhang, Y., Yu, J., Yu, Y., Zhang, F., Zhang, Z., Wu, A., Yan, X., Zhou, Y. and Wang, F. (2012). Slc39a7/zip7 plays a critical role in development and zinc homeostasis in zebrafish. *PLoS ONE* **7**, e42939. doi:10.1371/journal.pone.0042939

Zafar, N. and Khan, M. A. (2019). Growth, feed utilization, mineralization and antioxidant response of stinging catfish *Heteropneustes fossilis* fed diets with different levels of manganese. *Aquaculture* **509**, 120-128. doi:10.1016/j.aquaculture.2019.05.022

Zafar, N. and Khan, M. A. (2020). Effects of dietary iron on growth, haematology, oxidative stress and hepatic ascorbic acid concentration of stinging catfish *Heteropneustes fossilis*. *Aquaculture* **516**, 734642. doi:10.1016/j.aquaculture.2019.734642

Zhang, T., Guan, P. P., Liu, W. Y., Zhao, G., Fang, Y. P., Fu, H., Gui, J. F., Li, G. L. and Liu, J. X. (2020). Copper stress induces zebrafish central neural system myelin defects via WNT/NOTCH-hoxb5b signaling and pou3f1/fam168a/fam168b DNA methylation. *Biochim. Biophys. Acta Gene Regul. Mech.* **1863**, 194612. doi:10.1016/j.bbagrm.2020.194612

Zhao, L., Xia, Z. and Wang, F. (2014). Zebrafish in the sea of mineral (iron, zinc, and copper) metabolism. *Front. Pharmacol.* **5**, 33. doi:10.3389/fphar.2014.00033

Zimmer, A. M., Dymowska, A. K., Kumai, Y., Goss, G. G., Perry, S. F. and Kwong, R. W. M. (2018). Assessing the role of the acid-sensing ion channel ASIC4b in sodium uptake by larval zebrafish. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **226**, 1-10. doi:10.1016/j.cbpa.2018.06.012

Zimmer, A. M., Pan, Y. K., Chandrapalan, T., Kwong, R. W. M. and Perry, S. F. (2019). Loss-of-function approaches in comparative physiology: is there a future for knockdown experiments in the era of genome editing? *J. Exp. Biol.* **222**, jeb175737. doi:10.1242/jeb.175737