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REVIEW
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Ontogeny of the Osmoregulatory Capacity of Teleosts and the Role of Ionocytes

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Whilst osmoregulation in the adult teleost fish has been extensively studied and significant advances have been made in recent years, much less information exists regarding osmoregulation during the early stages of development of teleosts. Adult fish maintain their blood osmolality in a narrow physiological range, i.e., ≈ 280–360 mOsm kg^{-1}, through the combined osmoregulatory capabilities of several sites i.e., branchial chambers, skin, digestive system and urinary organs. However, embryonic and post-embryonic stages maintain their blood osmolality in a less narrow range of ≈ 240–540 mOsm kg^{-1} and osmoregulatory capacity is restricted to the cutaneous ionocytes located on the tegument with a transference in osmoregulatory function occurring during the early life stages to the developing digestive tract, the urinary organs and the developing branchial tissues and the ionocytes which they support. This review will discuss the development of osmoregulatory capacity that occurs throughout early life stages of teleosts and its role in conserving physiological homeostasis, focusing on the form and function of related mechanisms, i.e., the ionoregulatory cell or ionocyte, outlining the different roles and functions of different ionocyte types relative to their environment, i.e., freshwater or seawater, their plasticity and discuss spatio-temporal changes in ionocyte distribution that occur during ontogeny.

Keywords: osmoregulation, adaptability, larvae, embryos, early life stages, salinity, chloride cell, mitochondria rich cell

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INTRODUCTION

Prunet and Bornancin (1989; p. 92) describe teleost fishes as "an open system in dynamic equilibrium with aquatic surroundings." As osmoregulators, teleosts are homeo-isosmotic, i.e., able to regulate the concentration of solutes and their total osmolarity of their internal fluids at levels different to their external environment. Hence their body fluids remain relatively constant in spite of alterations to their external medium. They are, therefore, able to maintain their blood osmolality in a 280–360 mOsm kg^{-1} range, at the equivalent of 10–12 ppt (Evans et al., 2005). Hyper-osmotic regulators (most freshwater teleosts), subject to passive osmotic influx of water and diffusional loss of ions, mainly Na^+ and Cl^-, maintain body fluid concentration above that of their external surroundings. Hypo-osmotic regulators (most marine teleosts), subject to passive osmotic loss of water and diffusive gain of ions, maintain body fluid concentration below that of their external medium (**Figure 1**). Therefore, when faced with variations in external salinity, fishes must compensate for body fluid disturbances with a regulative capacity to adapt

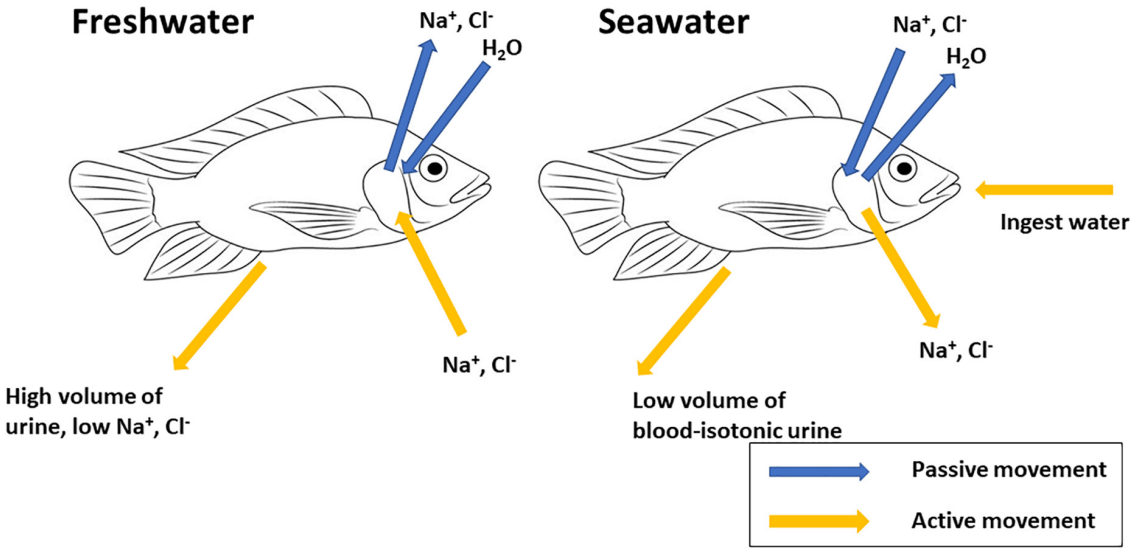

FIGURE 1 | Generalized schematic representation of mechanisms of osmoregulation in teleost fishes. Freshwater fishes are hyper-osmotic to their aquatic environment; compensatory mechanisms include active uptake of NaCl across gill epithelium, intestinal absorption of NaCl and excretion of large volumes of dilute urine. Marine teleosts are hypo-osmotic to their aquatic environment; compensatory mechanisms include active secretion of NaCl across gill epithelium, ingestion of seawater, intestinal absorption of NaCl and water and excretion of small volumes of blood-isotonic urine.

their osmoregulatory and ion transport strategies dependant on their surrounding environment (Ruiz-Jarabo et al., 2015).

Fishes are the most taxonomically diverse group of vertebrates with >32,400 identified species (Froese and Pauly, 2012) and have evolved to occupy almost all types of natural waters, ranging from low-ionic strength fresh waters to those of salinities of 80–142 ppt (Kinne, 1964; Parry, 1966; Griffith, 1974; Alderdice, 1988). The question of whether fish evolved in seawater or freshwater is still subject to debate. Nelson's (2006) study argued that fishes evolved in a marine environment, invaded freshwater habitats, and subsequently reinvaded marine environments, based on their level of phylogenetic advancement, i.e., the most primitive orders consisting almost entirely of strictly marine species, the next moderately advanced orders consisting mostly of freshwater species and the remaining orders mostly composed of marine species. Conversely, Evans and Claiborne (2009) argued that the considerably lower osmolality of the internal fluids of teleosts (equivalent to 9 ppt) when compared to that of the marine environment, represented evidence for the origins of this clade of fishes from a freshwater or brackish water environment. This is supported by Vega and Wiens (2012) trait reconstructions based on living and fossil taxa. These authors suggested that all extant actinopterygians (ray-finned) fishes were derived from a freshwater ancestor. Inconclusive fossil records of fishes shed no further light (Halstead, 1985; Evans and Claiborne, 2009).

Some fishes are restricted to living in a narrow range of salinity (stenohaline) while others can adapt to and tolerate broad ranges of salinity (euryhaline). It is estimated that *c.* 40% (*c.* 15,000) of these inhabit freshwater during at least one phase of their life cycle (Bond, 1996). This euryhalinity can range from either compulsory, migratory events in the lifecycle of a fish, e.g., catadromous fishes which spend their pre-adult life in freshwater and return to spawn in the sea or, conversely, anadromous fishes which grow and mature in sea water but return to freshwater to spawn, to less clearly defined movements of fishes that occupy estuarine waters or coastal habitats and undergo regular and frequent variations in the salinity of the medium in which they inhabit. This ability to cope with salinity changes depends on their capacity to osmoregulate and plays an important role in defining species and developmental stage-specific distribution (Schreiber, 2001).

It is well established that teleost embryos and larvae are able to maintain osmotic and ionic gradients between their internal and external environments (Guggino, 1980a,b; Alderdice, 1988; Kaneko et al., 1995), although full adult osmoregulatory capacity is not reached in these early developmental stages as organs are under-developed or absent (Varsamos et al., 2005). Compared to adult teleosts, larvae are able to maintain their blood osmolality in a less narrow range of $\approx$ 240–540 mOsm kg^{-1}, and this adaptive ability is accomplished by an early acquisition of osmoregulatory mechanisms that are different from those in adult fish. The ontogenetic development of osmoregulatory capacity, moving from a somewhat limited *trans*-membrane particle exchange at a cellular level in the embryonic blastular stage, to the fully-functioning regulatory tissues in juvenile and adult, such as the renal complex, the gut and the branchial epithelium, is described succinctly by Alderdice (1988; p.225) as a process which displays "continuity, with increasing complexity." As part of this ontogenic process, teleost embryos and post-embryonic larvae are able to maintain osmotic and ionic gradients between their internal and external environments due to the presence of numerous extrabranchial, cutaneous ionocytes commonly observed on the abdominal epithelium of the yolk-sac and other body surfaces of fish embryos and larvae.

While osmoregulation in the adult teleost fish has been extensively studied and significant advances have been made in recent years (Evans, 1999; McCormick et al., 2013), much less information, however, exists regarding osmoregulation in the early stages of development (Holliday, 1965; Alderdice, 1988; Tytler et al., 1993; Schreiber, 2001; Evans et al., 2005; Varsamos et al., 2005; Bodinier et al., 2010). Recently, the availability of precisely staged young fish, due to both the improved rearing methods by aquaculture and less stressful capture techniques for wild populations, has contributed to developments in the field (Evans et al., 2005). In addition, the development and application of new immunological techniques allowing visualization of delicate early life stages, has allowed the progression of ontogenetic studies.

THE IONOREGULATORY CELL OR IONOCYTE

Introduction

As opposed to movement of gases, ion movements require specific carriers and this "metabolic machinery" (Rombough, 2004) is found in a specific cell type, the ionocyte. Numerous work, dedicated to the study of their form and function in the adult teleost, has established that these cells are the primary extra-renal site responsible for the *trans*-epithelial transport of ions in adults and juvenile teleosts (Laurent and Dunel, 1980; Laurent, 1984; Perry et al., 1992; McCormick, 1995; Evans, 1999; Evans et al., 2005; Hiroi and McCormick, 2012).

Large spherical cells with eosinophilic granules were first described by Keys and Willmer (1932) of the Physiological Laboratory, Cambridge (United Kingdom) as "chloride-secreting cells," based on observations of the chloride secretory activity of gills of the adult eel (*Anguilla anguilla*) in seawater. The abbreviated name "chloride cell" is probably attributable to Copeland (1948) and was later clarified by Foskett and Scheffey (1982), who confirmed active transport of chloride ions by these cells using vibrating probe experiments on the opercular epithelium of sea-water adapted tilapia *Oreochromis mossambicus*. The term "mitochondria-rich cells" was first introduced by Lee et al. (1996), in order to emphasize the multifunctionality of the cells, i.e., they do more than just excrete chloride ions in seawater adapted fish. The term "ionocytes" was first introduced by Watrin and Mayer-Gostan (1996) in their study of ionoregulatory sites in the turbot (*Scophthalmus maximus*) and is currently accepted to be the most widely applicable term. Therefore, throughout this review, the term "ionocyte" will be used.

General Structure of Ionocytes

There are extensive reviews dealing with structure of ionocytes, e.g., Pisam and Rambourg (1991), McCormick (1995), Evans et al. (2005), Hwang and Lin (2013), Marshall (2011), and Dymowska et al. (2012). They have a number of common features that distinguish them from surrounding cells, sharing a specific complement of transporter or channels on the apical and basolateral membranes that allow directional movement of ions (Dymowska et al., 2012).

Ionocytes are highly specialized, polarized cells of large, columnar/ovoid shape displaying distinct ultra-structural features characteristic of ion-transporting cells, i.e., large numbers of mitochondria and a dense, tubular network that is continuous with the basolateral membrane causing extensive invagination (Doyle and Gorecki, 1961; Philpott, 1966). This tubular-vesicular system extends throughout most of the cytoplasm, and is closely associated with the mitochondria (Philpott, 1980; Laurent, 1984; Wilson et al., 2000a,b). It results in a large surface area for the placement of transport proteins, most importantly the ion-translocating enzyme Na^+/K^+-ATPase or "sodium pump" (García-Ayala et al., 1997) that has a role in both ion uptake and salt secretion in ionocytes in the teleost gill (Hiroi and McCormick, 2012). Early experiments confirmed labeled Na^+ and Cl^- efflux activity in live eels with the use of radioactive ouabain (a Na^+/K^+-ATPase inhibitor), thus inferring a basolateral location for the transporter protein Na^+/K^+-ATPase in mitochondria-rich cells (Silva et al., 1977). Subsequent work established that fish gill epithelia expressed large quantities of Na^+/K^+-ATPase (NKA) whose activity was usually proportional to the external salinity (De Rengis and Bornancin, 1984; McCormick, 1995). This has been attributed to an increased α-subunit mRNA abundance (Madsen et al., 1995; Singer et al., 2002) and protein amount (Lee et al., 2000; Tipsmark et al., 2002; Lin et al., 2003) or both (D'Cotta et al., 2000; Lin and Hwang, 2004).

Ion Transport in Seawater

Morphology

As a general rule, ionocytes in seawater or seawater-adapted fishes have the following morphological characteristics: the apical membrane is recessed below the surface of the surrounding pavement cells to form a concave pore or "crypt" that can be shared by accessory cells (ACs) (Karnaky, 1986), often forming "multi-cellular complexes" with cytoplasmic processes of accessory cells (ACs) extending into the apical cytoplasm of ionocytes to form complex interdigitations (Laurent, 1984; Wilson and Laurent, 2002) (see Section "Accessory Cells" below). These two types of cells share a single-stranded, "shallow" junction, suggesting a "leaky" pathway is present between the cells (Laurent, 1984; Hwang, 1988), thus providing a paracellular route for sodium extrusion (Sardet et al., 1979; Laurent, 1984).

Accessory Cells (ACs)

Hootman and Philpott (1980) first named the undifferentiated ionocytes found beside mature ionocytes in seawater flounder "accessory cells" or ACs. They appeared to be structurally analogous to ionocytes, in that they possessed large amounts of mitochondria and a labyrinthal tubular system, but were smaller and less developed than ionocytes with a less developed tubular system and lower expression of Na^+/K^+-ATPase relative to mature ionocytes. It has been reported that either a single or more than one accessory cell (AC), cluster around an ionocyte forming a "multi-cellular complex" (MCC) with a shared apical crypt (Hwang, 1988). ACs are small, semi lunar or pear-shaped

cells with lateral cytoplasmic processes that extend from the ACs to penetrate the apical portion of the adjacent ionocyte, sharing the apical cavity. ACs share a single-stranded, shallow junction with an ionocyte, suggestive of a "leaky" paracellular pathway thus giving additional paracellular pathways for the secretion of excess Na^+ from body fluids (Evans, 1999).

Na-Cl Secretion

Studies on mechanisms of Na-Cl secretion in seawater type ionocytes have been studied for several decades (Hsu et al., 2014) and the current and well accepted model for active NaCl transport by ionocytes in seawater adapted teleosts consists of three major ion-transporting proteins, i.e., Na^+/K^+-ATPase (NKA), $Na^+/K^+/2Cl^-$ co-transporter 1 (NKCC1) and a Cl-channel homologous to the human cystic fibrosis transmembrane receptor (CFTR) (Evans, 1999; Hirose et al., 2003; Evans et al., 2005; Hwang and Lin, 2013).

Briefly; basolateral Na^+/K^+-ATPase driven extrusion of three Na^+ from the cell to the plasma and entry of two K^+ into the cell then generates an electrochemical gradient that drives Na^+, coupled with Cl^- and K^+, back from the plasma into the cell's cytoplasm, via the $Na^+/K^+/2Cl^-$ co-transporter or (NKCC). NKCC therefore mediates the movements of Na^+, K^+ and Cl^- across the basolateral membrane of ionocytes and has a key role in cell volume homeostasis, maintenance of the electrolyte content and transepithelial ion and water movement in polarized cells (Cutler and Cramb, 2002). K^+ therefore enters the cell basolaterally both via the Na^+/K^+-ATPase and the NKCC co-transporter and is removed basolaterally from the cell via the potassium or K^+ channel. Cl^- exits the cell via an apical Cl^- anion channel or CFTR (cystic fibrosis transmembrane receptor).

Na^+ moves through the leaky paracellular pathway between ionocytes and ACs via a cation-selective paracellular pathway (Degnan and Zadunaisky, 1980), due to the negative potential created by transcellular Cl^- flux (Sardet et al., 1979). This transepithelial electrical potential across the gill epithelium drives Na^+ across leaky junction between ionocytes and accessory cells (Hsu et al., 2014) (see section "Accessory Cells (ACs)" above).

Ion Transport in Freshwater

Morphology

Ionocytes in freshwater usually lack an apical crypt and have their apical surfaces forming microvilli above the adjacent PVCs, which is consistent with their ion absorptive nature (Hwang, 1988; Perry et al., 1992; Marshall et al., 1997). However, an invaginated, crypt-like structure has been reported in ionocytes of the euryhaline Mangrove killifish (*Rivulus marmoratus*) in 1 ppt (King et al., 1989) and a slightly invaginated apical opening in the β – ionocytes in the freshwater adapted guppy (*Lebistes reticulatus*) (Pisam et al., 1987) and the loach (*Cobitis taenia*) and the gudgeon (*Gobio gobio*) (Pisam et al., 1990). This has similarly, been reported in freshwater adapted Tilapiine species e.g., the Mozambique tilapia (*Oreochromis mossambicus)* (Lee et al., 1996; van der Heijden et al., 1997; Uchida et al., 2000; Inokuchi et al., 2009) and the Nile tilapia (*Oreochromis niloticus*) (Pisam et al., 1993). The basolateral tubular system is less well developed in freshwater than in seawater adapted ionocytes, and they form extensive tight, multi-stranded junctions with adjacent PVC cells (Hwang, 1988).

Uptake Mechanisms

The ion-uptake mechanisms in freshwater fishes are more complicated, and both mechanism and ionocyte sub-types appear to vary amongst species (Hwang and Lin, 2013), remaining a source of contention (Dymowska et al., 2012; Hiroi and McCormick, 2012; Hwang and Lin, 2013; Hsu et al., 2014; Breves et al., 2020).

Several ion pumps, transporters and channels which are selectively expressed, either apically or basolaterally in the cell, are responsible for the ion-transport functions of the ionocytes (Hiroi and McCormick, 2012). It would be true to say that, over the last decades, a number of types and sub-types have been proposed, many of them unique to the species in which they were investigated, i.e., in various teleosts species (Doyle and Gorecki, 1961); guppy (*Lebistes reticulatus*) (Pisam et al., 1987); brown bullhead (*Ictalurus nebulosus*) (Goss et al., 1992; Goss and Perry, 1994); Japanese eel (*Anguilla japonica*) (Wong and Chan, 1999); Rainbow trout (*O. mykiss*) (Galvez et al., 2002; Reid et al., 2003); Medaka (*Oryzias latipes*) (Kang et al., 2008, 2010; Wu et al., 2010; Lin et al., 2012; Hsu et al., 2014); Zebrafish (*Danio rerio)* (Lin et al., 2006; Wang et al., 2009; Chang and Hwang, 2011; Hwang et al., 2011; Dymowska et al., 2012; Chang et al., 2013; Hwang and Chou, 2013; Hwang and Lin, 2013); tilapia (Oreochromis spp.) (Chang et al., 2001, 2003; Hiroi et al., 2005, 2008; Inokuchi et al., 2008, 2009); Nile tilapia (*O. niloticus*) (Fridman et al., 2013b); Mozambique tilapia (*O. mossambicus*) (Lee et al., 1996, 2000); killifish (*Fundulus heteroclitus*) (Copeland, 1948; Burns and Copeland, 1950; Wood and Marshall, 1994; Marshall et al., 1997; Katoh et al., 2001, 2003; Wood and Laurent, 2003; Hiroi et al., 2005, 2008; Laurent et al., 2006; Breves et al., 2020); Japanese seabass (*Lateolabrax japonicus*) (Inokuchi et al., 2017); seabass (*Dicentrarchus labrax*) (Blondeau-Bidet et al., 2019).

These different models can be seen to reflect the evolution of multiple ion uptake strategies from distinct species (Hwang and Lin, 2013) from fluctuating and diverse freshwater environments with varying ion compositions (Dymowska et al., 2012; Takei et al., 2014; Yan and Hwang, 2019) as well as the criteria used to identify and/or techniques used to visualize and functionally analyze the ionocytes and their transporters (Hsu et al., 2014). It is for this reason that the ability to define a definitive or comprehensive model of ion uptake mechanism in teleost is difficult (Hwang et al., 2011; Hwang and Lin, 2013).

Plasticity in Ion Transporting Function of Ionocytes

It is well established that changes in environmental salinity causes replacement of pre-existing ionocytes by newly differentiated ionocytes with a different ion-transport function (Wilson et al., 2000a; Tang et al., 2011; Christensen et al., 2012; Hiroi and McCormick, 2012; Breves et al., 2020). Recent advances in immunohistochemistry and complementary imaging techniques has identified not only freshwater and seawater specific isoforms of the Na^+K^+-ATPase alpha subunit but also the various ion-transporting proteins in the apical and basolateral membranes

TABLE 1 | Reports on the presence of extrabranchial ionocytes during embryonic and post-embryonic stages of commercially important teleosts.

Common name	Species	References
Plaice	*Pleuronectes platessa*	Shelbourne (1957); Roberts et al. (1973)
Pacific sardine	*Sardinops caerulea*	Lasker and Threadgold (1968)
Puffer	*Fugu niphobles*	Iwai (1969)
Guppy	*Poecilia reticulata*	Depeche (1973)
Killifish spp.	*Fundulus heteroclitus* and *Fundulus bermudae*	Guggino (1980a); Katoh et al. (2000); Breves et al. (2020)
Anchovy	*Engraulis mordax*	O'Connell (1981)
Ayu, flounder and carp	*Plecoglossus altivelis*, *Kareius bicoloratus*, *Cyprinus carpio*	Hwang (1989)
Mozambique tilapia	*Oreochromis mossambicus*	Ayson et al. (1994); Hwang et al. (1994); Shiraishi et al. (1997); Hiroi et al. (1999, 2005, 2008); Li et al. (1995); van der Heijden et al. (1997, 1999); Kaneko and Shiraishi (2001); Lin and Hwang (2004); Yanagie et al. (2009)
Yurbot	*Scophthalmus maximus*	Tytler and Ireland (1995)
Herring	*Clupea harengus*	Wales and Tytler (1996); Wales (1997)
Japanese eel	*Anguilla japonicus*	Sasai et al. (1998)
Japanese flounder	*Paralichthys olivaceu*	Hiroi et al. (1998)
Seaweed pipefish	*Syngnathus schlegeli*	Watanabe et al. (1999)
Rainbow trout	*Oncorhynchus mykiss*	Rombough (1999)
Orange spotted grouper	*Epinephelus coioides*	Caberoy and Quinitio (2000)
Sea bass	*Dicentrarchus labrax*	Varsamos et al. (2001); Varsamos et al. (2002a,b); Sucre et al. (2011)
Tilapia *spp*.	*Tilapia zillii*, *Oreochromis aureus*, *Oreochromis niloticus*, *Tristramella sacra*, *Sarotherodon galilaeus*	Fishelson and Bresler (2002)
Gilthead sea bream	*Sparus auratus*	Bodinier et al. (2010)
Nile tilapia	*Oreochromis niloticus*	Fridman et al. (2011, 2013a,b); Fridman, 2011; Melo et al. (2019)
Medaka	*Oryzias latipes*	Hsu et al. (2014)
Lake Van fish	*Alburnus tarichi*	Oguz (2018)
Yellowfin tuna	*Thunnus albacares*	Kwan et al. (2019)
White seabass	*Atractoscion nobilis*	Finnerty (2019)

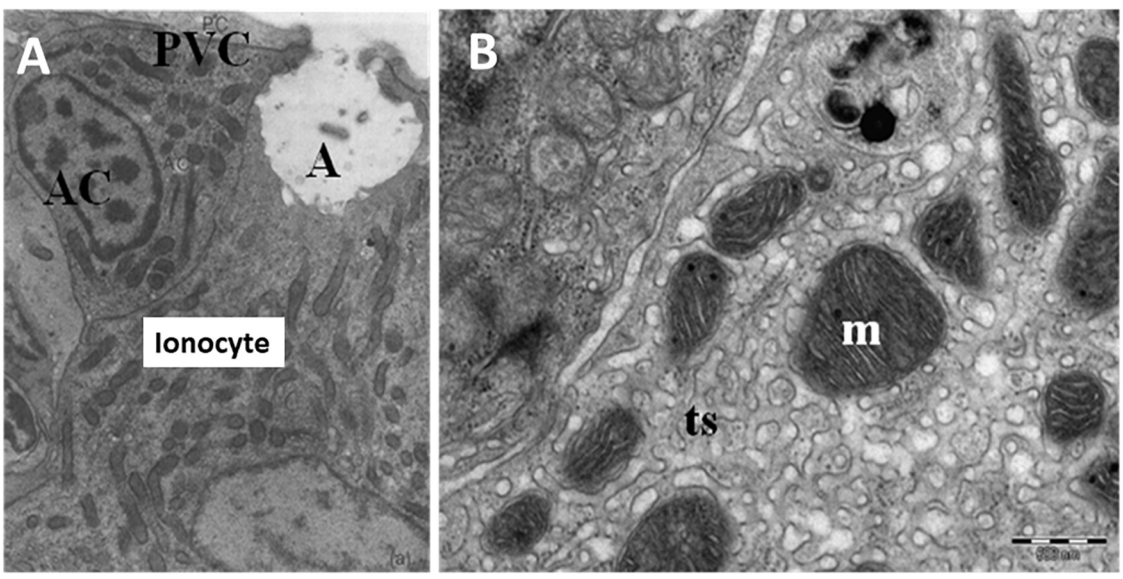

FIGURE 2 | Ultrastructure of ionocytes in freshwater adapted *Oreochromis niloticus* larvae. **(A)** A multicellular complex (MCC) formed by a mature ionocyte and an accessory cell (AC) sharing a single apical crypt **(A)** lying beneath a pavement cell (PVC). Reduced osmium staining; x 11,900 and **(B)** Detail of mitochondria with tubular system (m; mitochondria, ts; tubular system) (Bar = 500 nm) (Fridman, 2011).

of ionocytes, leading to the understanding that ionocytes display a plasticity of function in terms of ion transport which is determined by the localization of the various ion-transporting proteins in the apical and basolateral membranes (Inokuchi et al., 2017). This has been correlated with studies showing the plasticity of genes encoding sub-cellular effectors of ion transport expressed during salinity acclimation (Scott et al., 2004; Fiol and Kültz, 2007).

IONIC AND OSMOTIC BALANCE IN EGGS AND DURING GASTRULATION

Leading up to ovulation, the transfer of nutrients and ions occur through the contact between oocyte and follicular cell microvilli and, therefore, their ionic and osmotic control are a function of the parental regulatory system (Alderdice, 1988). At ovulation or release from the follicular cells, the mature eggs become free in the ovary of the adult and, surrounded by ovarian fluid, are still under the control of the adult regulatory system. During this period their plasma membrane appears to be relatively permeable to water and responds to changes in the ovarian fluid (Sower and Schreck, 1982); osmotically the ovarian fluid is very similar to the blood plasma (Hirano et al., 1978) and the blood plasma is in physiological balance with the external environment (Sower and Schreck, 1982).

At spawning, the mature eggs are hypotonic to sea water and hypertonic to fresh water. Independent regulatory capacity is first evident with activation of the embryo occurring in teleosts at metaphase II, the stage of meiosis following the

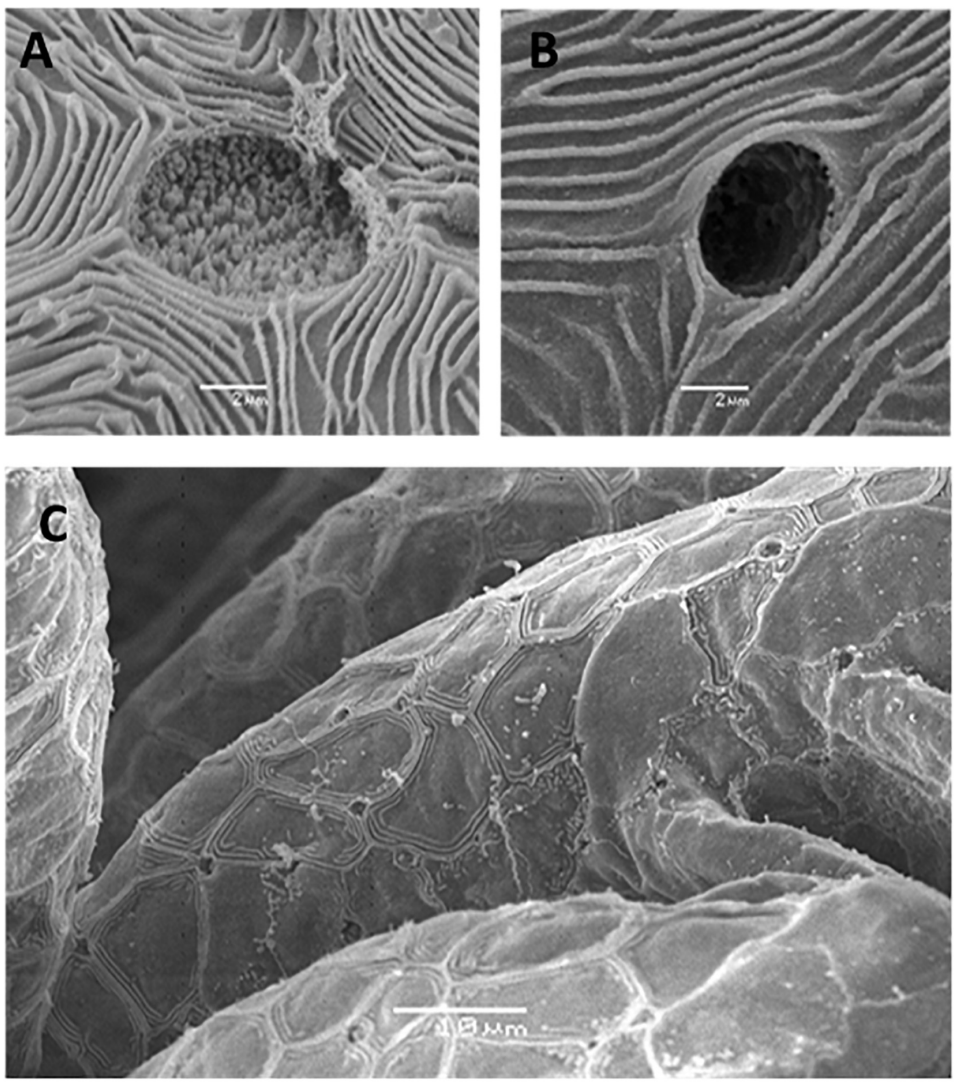

FIGURE 3 | Scanning electron micrographs of external morphology of ionocytes during early life stages. **(A)** Apical opening of an ionocytes on yolk-sac epithelia of Nile tilapia in freshwater adapted larvae at hatch (Bar = 2 μm), **(B)** Apical opening of an ionocyte on yolk-sac epithelia of Nile tilapia in brackish water adapted larvae at hatch (Bar = 2 μm) and **(C)** Lower magnification of apical openings of an ionocyte on gill filaments of freshwater larvae at 3 dph (Bar = 10 μm) [from Fridman et al. (2011)].

extrusion of the polar body. During activation, the cortical alveoli, underlying the oocyte plasma membrane, discharge their contents into the presumptive perivitelline space between the chorion and the plasma membrane, by a process called cortical alveolar exocytosis, causing an uptake of water from the external environment across the chorion, lifting it away from the plasma membrane by displacement and blocking the micropyle therefore preventing polyspermy. Subsequent regulation and maintenance of the integrity of the egg appears to be achieved by the resistive maintenance of a tight plasma membrane and limited *trans*-membrane water and ion fluxes (Bennett et al., 1981).

Following this is the transitory developmental blastula stage, characterized by the formation and development of the blastoderm or overgrowth of the yolk by a single layer of cells called a blastomere, which spreads out as a flat plate over the upper surface of the yolk mass. There is little evidence to suggest that there is much control over water and ion exchange between egg and external environment at this stage and any regulatory capacity that does exist is presumed to arise from low *trans*-membrane fluxes and appears to be "neither modulated nor selective" (Alderdice, 1988; p. 241). Indeed, Alderdice (1988) concludes that the establishment of osmotic or systemic regulation, begins during gastrulation, and is in place by yolk-plug closure; an increase in the permeability of the plasma membrane during gastrulation coincides with the appearance of integumental or cutaneous ionocytes on the epithelium of the body surface and yolk-sac of the developing embryo, marking the start of the selective restriction of ions and water transfer or active ionoregulation (Guggino, 1980b). A recent study by Dahlke et al. (2020) of homeostatic regulation

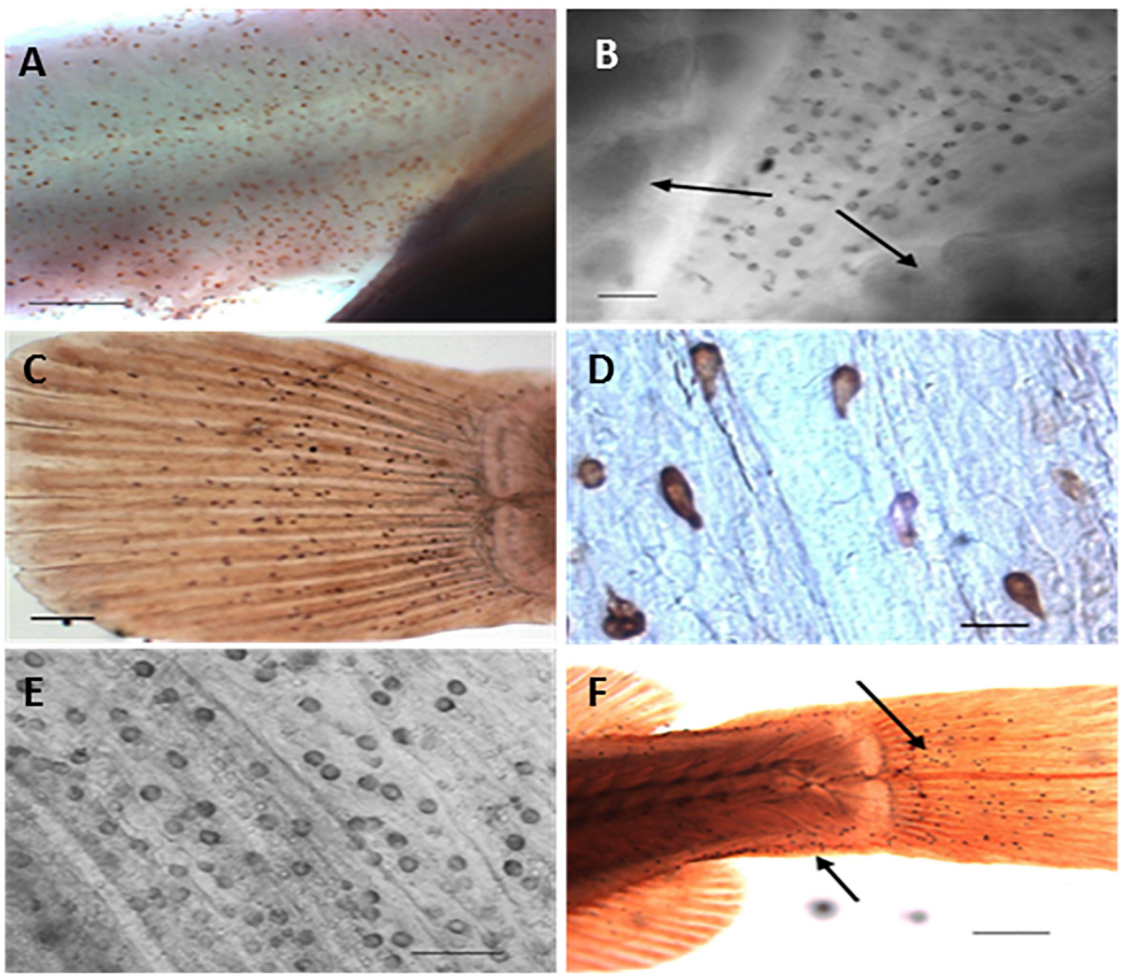

FIGURE 4 | Distribution of ionocytes as revealed by anti-Na^+/K^+-ATPase antibody during post-embryonic development of Nile tilapia (*Oreochromis niloticus*) using light microscopy. **(A)** Detail of anal region of freshwater adapted larvae at 3 days post hatch (dph showing clustered immunoreactive ionocytes (Bar = 200 μm), **(B)** Ionocytes on ventral region of brackish water adapted larvae at 3 dph. Arrows indicates presence of gills underlying opercula (Bar = 30 μm), **(C)** Caudal fin of freshwater adapted larvae at 3 dph showing immunoreactive ionocytes (Bar = 200 μm) (LM), **(D)** Detail of immunoreactive ionocytes on caudal fin of brackish water adapted larvae at 3 dph (Bar = 20 μm), **(E)** Inner opercular area of freshwater adapted larvae at 5 dph showing immunoreactive ionocytes (Bar = 50 μm) (LM) and **(F)** Caudal extremity of brackish water adapted larvae at 7 dph. Arrows indicate location of clustered immunoreactive ionocytes (Bar = 300 μm) [from Fridman et al. (2011)].

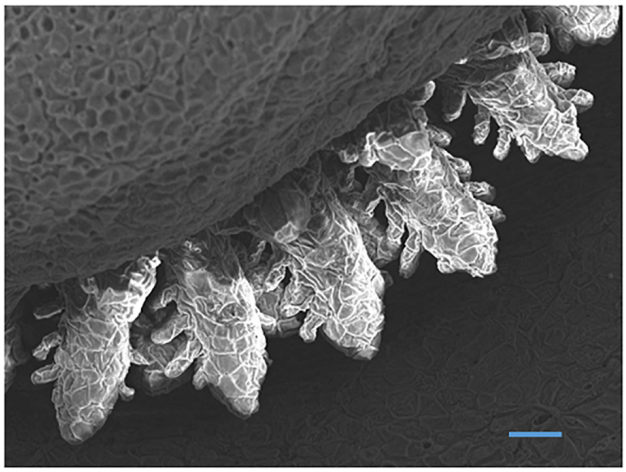

FIGURE 5 | Scanning electron micrograph of developing gills in yolk-sac larvae of Nile tilapia at hatch showing filaments with budding secondary lamellae (Bar = 50 μm) (Fridman, 2011).

in embryo Atlantic cod (*G. morhua*) implies that the gastrulation period represents a critical transition from maternal control to active ionic regulation. Epiboly, or cellular overgrowth of the yolk and pericardial regions of the embryo, occurs when the developing ectodermal layer of the blastoderm, along with the marginal ridge of the blastodisc and its inner layer or "germ ring" grows to form an epiblast. This, combined with the periblast, which is the initial covering of the yolk, forms the yolk sac. The opening called the yolk-plug or blastopore overgrows when gastrulation is complete.

Recent evidence suggests the aquaporins, small, hydrophobic integral membrane channel proteins that aid the passive movement of water across bilaminar membranes against an osmotic gradient (Cerdà and Finn, 2010), have a vital adaptive role in maintain homeostasis during oocyte development and embryogenesis (see Cerdà et al., 2017).

IONOCYTES DURING EARLY LIFE STAGES

The Cutaneous or Extrabranchial Ionocyte

After hatch, post-embryonic larvae are able to live in media whose osmolality differs from their own blood osmolality, and this tolerance is based on ability to osmoregulate. This is due to the presence of numerous cutaneous ionocytes commonly observed in the yolk-sac membrane and other body surfaces of fish embryos and larvae, i.e., head, trunk and fins. These extrabranchial cells are considered to play a definitive role in osmoregulation during early development by secreting and absorbing ions in seawater and freshwater environments, respectively, until the time when gills become fully developed and branchial ionocytes become functional (Kaneko and Shiraishi, 2001). The flat surface that these sites offer has allowed repeated morphological analyses or analysis of ion fluxes that branchial surfaces, with their complex three-dimensional nature, have precluded and offer a convenient experimental substitution for branchial ionocytes thus shedding light on their morphology, function and differentiation (Hiroi and McCormick, 2012).

The first report of localization of ionoregulation to the integument of teleost larvae was that of Shelbourne (1957) who investigated chloride regulation sites in marine plaice larvae (*P. platessa*). Subsequent and similar reports are summarized in **Table 1**.

In general, embryonic and larval integumental ionocytes appear structurally and biochemically similar to adult branchial ionocytes (**Figure 2**). Ayson et al. (1994) using transmission electron microscope to examine ionocytes in the yolk-sac membrane of freshwater and seawater-adapted *O. mossambicus* tilapia embryos and larvae noted a similarity with ionocytes in branchial and opercular epithelium of the adult fish; the cytoplasm of the ionocytes was seen to contain numerous mitochondria and Na^+/K^+- ATPase located on the extensive and well-developed tubular system. In addition, SEM indicated clear changes in the size and structure of apical openings in integumental ionocytes as a response to changes in salinity, as displayed in adults (**Figure 3**). Correspondingly, van der Heijden et al. (1999), using immunostaining of cross sections of whole tilapia larvae (*O. mossambicus*) with an antibody against α -subunit of Na^+/K^+- ATPase, found extrabranchial ionocytes (from 24 h post-hatch onward) in both freshwater and seawater adapted larvae to be ultrastructurally similar to that of ionocytes in the branchial epithelium of adult fish. In addition, Shiraishi et al. (1997) reported the presence of MCCs (see Section "Accessory Cells" below) in the yolk-sac membrane of seawater–adapted tilapia larvae *O. mossambicus*.

Integumental Ionocytes During Embryonic and Post-embryonic Stages

The first appearance of ionocytes in fish embryos was reported on the yolk-sac epithelia of dechorionated Mozambique tilapia (*O. mossambicus*) embryos as early as 26 h post-fertilization, but no apical crypt to indicate functionality was apparent until 48 h post-fertilization (Lin et al., 1999). Similarly, Ayson et al. (1994), using SEM and TEM, observed ionocytes distributed underneath the pavement cells on the yolk-sac epithelium of Mozambique tilapia (*O. mossambicus*) embryos at 30 h post-fertilization in both freshwater and seawater but were presumed to be not yet functional as no apical openings were noted. Apical openings of ionocytes were first observed, albeit at a low density, at 48 h post-fertilization or half-way to hatching.

The site of active ionoregulation in the integument of post-hatch or post-embryonic teleost larvae was first demonstrated by Shelbourne (1957) who investigated the chloride regulation sites in the European plaice larvae (*P. platessa*) and, since then, integumental ionocytes have been reported in the post-embryonic stages of many commercially important species (see **Table 1**). There exists a distinct spatial shift in ionocyte distribution in both freshwater and marine teleosts; it is generally accepted that integumental ionocytes are initially

responsible for osmoregulation prior to development of the adult osmoregulatory organs in *O. mossambicus* (Ayson et al., 1994; Shiraishi et al., 1997; Hiroi et al., 1999), *O. niloticus* (Fridman et al., 2011; see **Figure 4**) killifish (*F. heteroclitus)* (Katoh et al., 2000) and gilthead seabream (*S. auratus*) (Bodinier et al., 2010). The extrabranchial integument that can potentially be occupied by larval ionocytes comprises the yolk-sac, head, trunk and fins (Varsamos et al., 2005). Distribution of ionocytes in the integuments can also clearly be seen to be species dependant (Varsamos et al., 2005) and vary ontogenetically (Wales and Tytler, 1996; Fishelson and Bresler, 2002; Janicke et al., 2007; Bodinier et al., 2010).

Gill Development of the Role of Branchial Ionocytes During the Post-embryonic Period

A general feature of early fish larvae is the absence of fully developed gills (Segner et al., 1994), and the ontogeny of the gills forms an important part of the developmental process of the embryonic and larval fish. The sequence of gill development is described by Hughes (1984) as "continuous" with the epithelium that forms the surface of the gill arches becoming the surface of the filament and afterward the surface of the lamellae (**Figure 5**). Coinciding with this development is the maturation of other parts of the respiratory and cardiovascular system and coordination of the pumping systems for water and blood flow through the gills immediately prior to metamorphosis (Rombough, 2004; **Figure 6**).

It is widely accepted that gills in fish larvae have an ionoregulatory function before a respiratory function, however, the exact timing of ionocyte functionality in the fish gill is a matter of debate (Alderdice, 1988); less is known about the ontogeny of branchial ionocytes in fish larvae, with the majority of osmoregulatory studies in embryos and larvae focusing on integumental ionocytes. However, a clearly defined temporal staging of the appearance of ionocytes, conferring ability to cope with varying environmental conditions during early development, is evident throughout the yolk-sac period. An ontogenic transfer of regulative, osmoregulatory function

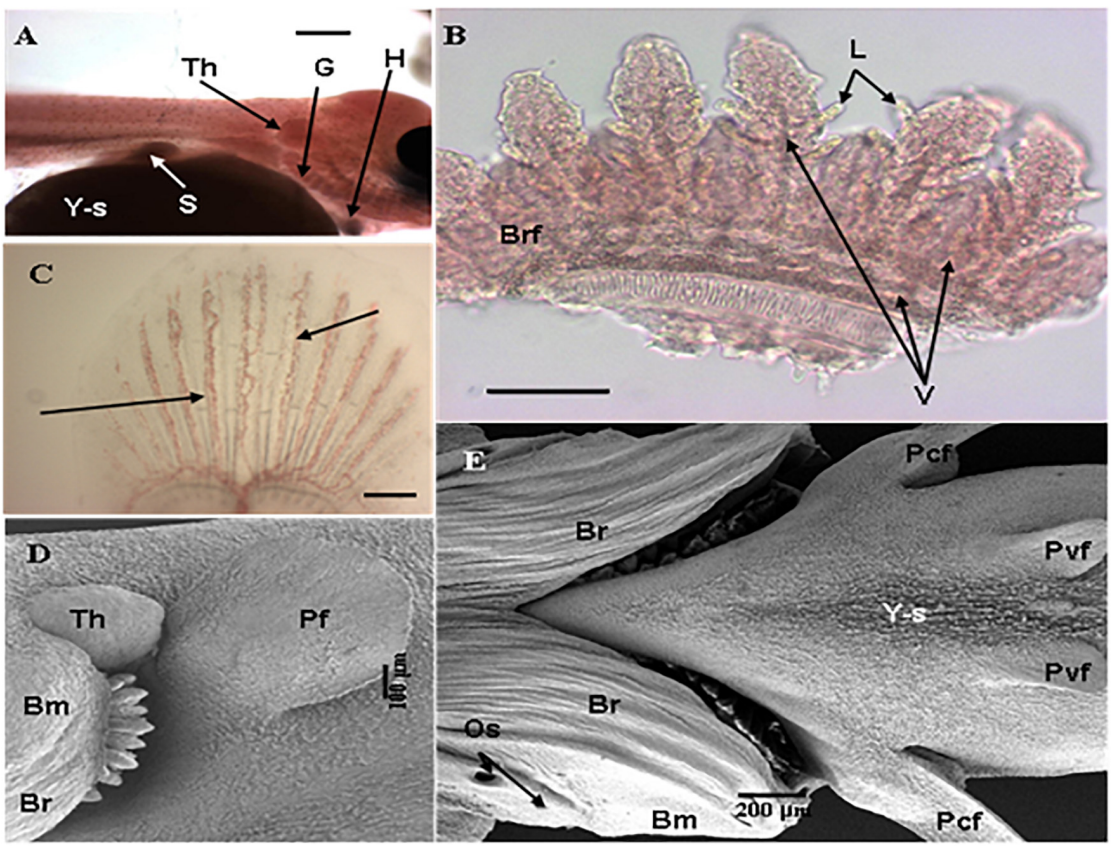

FIGURE 6 | Development of branchial system and vasculature in Nile tilapia. **(A)** Freshwater adapted larvae at 1 dph showing gills (G), budding thymus (Th), heart (H), yolk-sac (Y-s) and stomach (S) (Bar = 500 μm) (LM), **(B)** Detail of branchial arch of freshwater adapted larvae at 1 dph showing pairs of hemibranchs or branchial filaments (Brf) with emergent lamellae (L) with clearly defined vasculature (V) (arrows) (Bar = 100 μm) (LM), **(C)** Developing caudal fin of larvae adapted to brackish water at 3 dph showing vasculature (arrow) (Bar = 200 μm) (LM), **(D)** Freshwater adapted larvae 3 dph showing pectoral fin (Pf), prominent thymus (Th) and branchiostegal membrane or operculum with visible branchiostegal rays (Br) partly covering gill arches and developing gills (Bar = 100 μm) (SEM) and **(E)** Underside of brackish water adapted larvae at 7 dph showing gills completely covered by the fully-defined branchiostegal membrane (Bm) with branchiostegal rays (Br), opercular spiracles (Os) and pectoral (Pcf) and pelvic fins (Pvf) developing on shrunken yolk-sac (Y-s) (Bar = 200 μm) (SEM) [from Fridman et al. (2011)].

from the integumental system to the developing branchial epithelial sites, culminating in the fully-functioning, branchial ionocytes has been widely reported, i.e., the sea water flounder (*K. bicoloratus*) (Hwang, 1989), the summer flounder (*P. dentatus*) (Schreiber and Specker, 1998), the rainbow trout *O. mykiss* (Gonzalez et al., 1996; Rombough, 1999), the trout (*S. trutta*) (Rojo et al., 1997), the Japanese flounder (*P. olivaceus)* (Hiroi et al., 1998), the guppy (*P. reticulata*) (Shikano and Fujio, 1998a,b), the Nile tilapia (*O. niloticus*) (Fridman et al., 2011, 2013a,b), Mozambique tilapia (*O. mossambicus*) (Li et al., 1995; van der Heijden et al., 1999) and the killifish *(F. heteroclitus*) (Katoh et al., 2000), sea bream (*D. labrax*) (Varsamos et al., 2005; Bodinier et al., 2009) and the gilthead seabream (*S. auratus*) (Bodinier et al., 2010).

SUMMARY

This review has outlined the main physiological changes and adaptations in osmoregulatory capacity that occur throughout the early life stages of teleosts and the role of the ionoregulatory cell or ionocyte in conserving physiological homeostasis. It describes the different ionocyte types relative to their environment, i.e., freshwater or seawater, their plasticity in form and function and discusses spatio-temporal changes in integumental ionocyte distribution that occur during gastrulation and embryonic and post-embryonic stages, prior to transfer of full regulative function to the osmoregulatory organs of the adult teleost, i.e., branchial chambers, skin, digestive system and urinary organs.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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