Ontogenetic Changes in Blood Osmolality During the Postembryonic Development of Zebrafish (Danio rerio)

Guy Charmantier,1 Mai Nguyen-Chi,2 and Georges Lutfalla2

Abstract

The zebrafish Danio rerio is a teleost model species widely used in developmental genetics, biomedical studies, toxicology, and drug screening. Despite the interest of this species in research, little is known through indirect observations about its blood osmolality, which is a key parameter for diverse experiments. In this study, we directly measured blood osmolality using nano-osmometry at different stages of zebrafish postembryonic development. We found that blood osmolality is close to 240 mOsm·kg⁻¹ in early larvae. It progressively increased to ~270 mOsm·kg⁻¹ during the larval development before reaching ~300 mOsm·kg⁻¹ after metamorphosis in juveniles and later in adults. These ontogenetic changes in blood osmolality illustrate the physiological changes in osmoregulation associated with postembryonic development, including metamorphosis. These values are of practical interest for adjusting the osmolality of fixatives and cell and tissue culture media for research using zebrafish as a model.

Keywords: teleost, zebrafish, ontogeny, osmoregulation, nano-osmometry

Introduction

The zebrafish Danio rerio (Buchanan-Hamilton 1822) is a stenohaline freshwater teleost (Cyprinidae). Its main natural range covers the Ganges and Brahmaputra River basins in India, Bangladesh, and Myanmar where it is typically found in slow-moving or standing water bodies.1 Exported over a century ago, it became a common aquarium species easy to culture. This feature and other traits such as small size, rapid development, optical transparency of early embryos, availability of genetic databases, and applicability of molecular tools, have made the zebrafish a key model for developmental genetics, toxicology, drug screening, and biomedical studies, including cancer, host–pathogen interactions and regenerative medicine. The experimental use of this fish has been facilitated by descriptions of its embryonic2 and postembryonic3 stages of development, and of practical rules for its culture.4

Embryos are able to develop below 4%o (~110 mOsm·kg⁻¹),5 and embryos and early larvae can stand extremely low ion concentrations of 34 µM Na⁺, 40 µM Cl⁻ (salinity ~0.003‰; osmolality ~0.08 mOsm·kg⁻¹).6 In later stages of development, it has been shown that adult zebrafish can regulate plasma and whole-body electrolyte concentrations,7 from soft waters with low ion concentrations (Na⁺: 35 µM; Cl⁻: 43 µM) to hard water (Na⁺: 1480 µM; Cl⁻: 1625 µM), with corresponding osmolalities of 0.1 to 7–10 mOsm·kg⁻¹.

In freshwater, the zebrafish is submitted to a passive loss of ions, compensated by ion uptake from the external medium and limited by production of dilute urine, resulting in the regulation of the osmolality of the blood as in other...
freshwater teleosts. D. rerio has become a model for the study of epithelial transport associated with ionic and osmotic regulation. The ion-transporting cells or ionocytes, their structure, and sequential development from skin in embryos to gills in postembryonic stages, their different types, and their differentiation pathways, have been studied in embryos, in larvae, and in adults. Ionocytes are involved in ion transport, particularly in ion uptake from ion-poor media. Their functions, including endocrine control and gene expression, have been studied in adults and in early developmental stages.

Despite this wide interest over the past twenty years regarding the ionocyte structure and function in zebrafish, and despite the importance of this fish as a key species in research, little is known about the value of its blood osmolality, although it represents a basic parameter for diverse experiments. Very few data obtained through indirect observations are available on the actual level of the blood osmolality at each developmental stage. In addition to their scientific interest, knowing these values would yet be valuable from a practical point of view, for instance to precisely adjust the osmolality of fixatives, or for use in studies, we used the wild-type reference AB line (https://zfin.org/ZDB-GENO-960809-7).

Animals

Specimens of D. rerio were obtained from the culture facility of the University of Montpellier. For the present studies, we used the wild-type reference AB line (https://zfin.org/ZDB-GENO-960809-7).

In the laboratory, females were kept in 3.5 L polycarbonate aquaria connected to a closed recirculating system (Tecniplast), in which fresh water with addition of Instant Ocean salts at 0.06 g·L⁻¹ was adjusted at pH close to 7 by the addition of 10 mM CO₂ in a closed recirculating system. The water was changed every 2 days, and the trophic level of the aquarium was maintained with freshly hatched Artemia salina day eggs (Instant Ocean). The water temperature was held constant at 28°C ± 0.5°C throughout the experiments. To prevent phototoxicity, all fish were reared under artificial light and 12-hour light/dark cycles. The zebrafish embryos were reared under these conditions until the desired developmental stage, and the adults were kept under the same conditions. Animals were fed with live brine shrimp and flake food.

Table 1. Blood Osmolality (mOsm·kg⁻¹) Evaluated by Different Authors in Danio Rerio at Different Stages

| Embryo                  | Early develop.: Larva | Mid develop.: Juvenile | Adult               | Method                           | Reference |
|-------------------------|-----------------------|------------------------|---------------------|---------------------------------|-----------|
| Mid-blastula            |                       |                        |                     | Culture in different media      | 25        |
| Optimum 315            |                       |                        |                     | Assumption                      | 26        |
| Cell injection          |                       |                        |                    |                                 |           |
| recommended 300        |                       |                        |                    |                                 |           |
| Same osmolality         | Same osmolality       |                        |                    |                                 |           |
| Lower osmolality        | Lower osmolality      |                        |                    |                                 |           |
| Lower osmolality        | Lower osmolality      |                        |                    |                                 |           |
| Same osmolality         | Same osmolality       |                        |                    |                                 |           |
| Same osmolality         | Same osmolality       |                        |                    |                                 |           |
| Na⁺ & Cl⁻: 210 mM (soft water) |                |                        | Na⁺ & Cl⁻: 210 mM (soft water) | Plasma, ion chromatography | 21        |
| 260 mM (hard water)     |                       |                        |                    |                                 |           |
| 4–5 dpf                 |                       |                        |                    |                                 |           |
| 240                     |                       |                        |                    |                                 |           |
| 320                     |                       |                        |                    | Estimated from other studies     | 21        |

Soft water: 48 µM Na⁺ & Cl⁻; 4 µM Ca²⁺. Hard water: 3100 µM Na⁺ & Cl⁻; 3200 µM Ca²⁺. “Same” and “lower” osmolality: compared with adults in which blood osmolality is generally estimated at 320 mOsm·kg⁻¹. dpf, days postfertilization.
addition of 40 μL of NaOH 10 N per liter. Constant conditions in the laboratory were 0.4% salinity/4 mOsm·kg⁻¹ osmolality/400 μS·cm⁻¹ conductivity, a temperature of 28°C and a 12-h light:12-h dark cycle. The fish were fed twice per day. Newly hatched larvae were collected in Petri dishes and reared in glass vials (~300 mL) under the same conditions concerning water, temperature, and light. Larvae were fed from days 5.

Before experiments, the weight and length (SL [standard length], from snout to caudal peduncle) of 3-week-old and older individuals were measured. For staging the animals during their postembryonic life, we used the criteria based on externally visible anatomy according to Parichy et al. Following the hatching period at 48–72 hpt (hours post-fertilization), larvae develop until becoming juveniles following a metamorphic process.

During metamorphosis, the morphology, anatomy, and physiology of the larvae change, with loss or remodeling of larval features and acquisition of juvenile and adult features. Juveniles have most adult features in the absence of sexual maturity, with corresponding changes in behavior and ecology in the wild. In the culture conditions used in this study, the metamorphic transition between larva and juvenile occurred at ~6 weeks pf and the transition from juvenile to adult occurred at ~2–3 months pf.

The developmental stages tested in the osmoregulation experiment included stages from early larvae to adults. We conducted individual measurements of blood osmolality at the following stages: 3-week-old larvae (L 3wk), 6-week-old larvae/juveniles (L-Juv 6wk), 2-month-old and 3-month-old juveniles/adults (Juv-Ad 2m, Juv-Ad 3m), and 2-year-old and 3-month-old adults (Ad 2yr, Ad 2yr1m). As early larvae/juveniles, pools of 25–50 larvae were used. For staging the animals according to their size (SL): the mean blood osmolality was 270 mOsm·kg⁻¹ in the smaller larvae (SL: 7.4 ± 0.2 mm), not different from the value in 3-week-old larvae (p > 0.9999), but in the bigger 6-week-old larvae/juveniles (SL: 10.6 ± 1.3 mm), the mean blood osmolality was 300 mOsm·kg⁻¹, higher than in 3-week-old larvae (p < 0.001) (Table 2 and Supplementary Table S1).

Later in development, 2- and 3-month-old juvenile-adults had a mean blood osmolality of 300–304 mOsm·kg⁻¹, also higher than in 3-week-old larvae (p < 0.001 for 2-month-old juvenile-adults vs. 3-week-old larvae). Adult fish, 2-year-old and tested twice, yielded similar mean blood values, 302 and 303 mOsm·kg⁻¹ (p < 0.001 for 2-year-old vs. 3-week-old larvae, Supplementary Table S1).

**Statistical analysis**

Blood osmolality values (mean ± standard deviation) in adult males (302.4 ± 8.25 mOsm·kg⁻¹, n = 11) and females (303.2 ± 9.96 mOsm·kg⁻¹, n = 5) were not significantly different (Mann–Whitney test, two-tailed, p = 0.9789); therefore, values from both sexes were pooled.

Blood osmolality increased during the postembryonic development from larvae to juveniles and adults of D. rerio (Table 2 and Fig. 1). The lowest value was found in body fluids from homogenates of 5-dpf-old larvae, at 243 mOsm·kg⁻¹. Mean blood osmolality was 268 mOsm·kg⁻¹ in 3-week-old larvae and 285 mOsm·kg⁻¹ in a group of 6-week-old larvae/juveniles. In the latter, two subgroups were distinguished according to their size (SL): the mean blood osmolality was 270 mOsm·kg⁻¹ in the smaller larvae (SL: 7.4 ± 0.2 mm), not different from the value in 3-week-old larvae (p > 0.9999), but in the bigger 6-week-old larvae/juveniles (SL: 10.6 ± 1.3 mm), the mean blood osmolality was 300 mOsm·kg⁻¹, higher than in 3-week-old larvae (p < 0.001) (Table 2 and Supplementary Table S1).

**Discussion**

A cryoscopic nano-osmometer allows direct measurements of osmolality on very small volumes of liquid down to 30 nL. This technique was successfully used to measure the blood osmolality on individual zebrafish at different developmental stages, from 3-week-old larvae to juveniles and adults.

The blood osmolality of adult zebrafish was close to 300 mOsm·kg⁻¹. In a study of ionic regulation in this species, it was reported that total plasma Na⁺ and Cl⁻ concentration in adult zebrafish was 260 mM (or mEq·L⁻¹) in hard water at 3 mOsm·kg⁻¹, close to the water used in this study (Table 1). As Na⁺ and Cl⁻ build up ~90% of the total blood osmolality, these ion concentrations correspond to an estimated osmolality of ~290 mOsm·kg⁻¹, close to our own finding that is lower than the estimated value of 320 mOsm·kg⁻¹ (the “textbook value”) cited in a recent study. In other
Table 2. Ontogeny of Osmoregulation of Danio rerio, in Fresh Water at 28°C

| Osmolarity of FW mOsm·kg⁻¹ | Length mm SL Mean±SD; (n) | Length mm Min-max | Weight mg Mean±SD; (n) | Weight mg Min-max | Blood osmolality mOsm·kg⁻¹ Mean±SD; (n) | Blood osmolality mOsm·kg⁻¹ Min-max |
|-----------------------------|--------------------------|--------------------|------------------------|-------------------|----------------------------------------|----------------------------------|
| L 5d                        | 3.4±0.2; (12)            | 3.3–3.9            | 3.2±0.4; (11)          | 2.8–3.5           | 243 (±5) (4 pools of 300–500)          | 235–247                        |
| L 3wk                       | 5.9±0.3; (11)            | 5.1–6.4            | 8.1±4.5; (39)          | 3.2–22.9          | 268±11; (11)                          | 260–290                        |
| L-Juv 6wk                   | 9.0±0.2; (39)            | 6.9–12.2           | 11.2±4.3; (20)         | 6.0–22.9          | 285±18; (39)                          | 250–310                        |
| Subgroup SL >8 mm           | 10.6±1.3; (20)           | 8.7–12.2           | 4.8±1.2; (19)          | 3.2–7.8           | 300±8; (20)                           | 280–310                        |
| Juv-Ad 2m                   | 18±5; (10)               | 15–22              | 100±62; (10)           | 42–136            | 270±12; (19)                          | 250–290                        |
| Juv-Ad 3m                   | 21±3; (4)                | 18–24              | 141±55; (4)            | 78–193            | 304±6; (10)                           | 295–315                        |
| Adult 2yr                   | 33±3; (10)               | 30–37              | 782.9±317.6; (6)       | 531–1250          | 300±4; (4)                            | 295–305                        |
| Adult 2yr 1m                | 33.9±2.3; (6)            | 31.8–37.5          | 580.9±46.2; (4m)       | 302±5; (6)        | 303±10; (10)                          | 283–315                        |

Main stages and the main osmolality values are highlighted in bold.

Values of length, weight, and blood osmolality at different stages of postembryonic development. Mean±SD, with minimum–maximum range. For the weight results (fifth and sixth columns) in Adult 2yr 1m: n, total 6, including 4 males (m) and 2 females (f).

Osmolarity of body fluids.

L: Larva; Juv: Juvenile; Ad: Adult; wk: week; m: month; yr: year; SD, standard deviation.

Under culture conditions used in this study, metamorphosis started at a SL of 11.2±11.7 mm at the onset of metamorphosis. However, at this time, all individuals were still small, with a SL of 6.2–12.6 mm and a SL of 4–6 weeks postfertilization. The measured blood osmolality was lower than in adults, which is in agreement with previous indirect observations. Due to the small size of early larvae at 5 dpf preventing reliable sampling, individuals were considered as juveniles.

Later in development, in 3-week-old larvae, blood osmolality was significantly different from L-Juv 6wk and significantly different from L 5d, L 3wk, and L-Juv 6wk.

This value is however very close to that of 240 mOsm kg⁻¹ reported, all values compatible with our own findings.

In larval stages, we found through our measurements that blood osmolality was lower than in adults, which is in agreement with previous indirect observations. Due to the small size of early larvae at 5 dpf preventing reliable sampling, individuals were considered as juveniles.

Later in development, in 3-week-old larvae, blood osmolality was significantly different from L-Juv 6wk and significantly different from L 5d, L 3wk, and L-Juv 6wk.

This value is however very close to that of 240 mOsm kg⁻¹ reported, all values compatible with our own findings.
ONTOGENY OF BLOOD OSMOLALITY IN ZEBRAFISH

While emphasis is often put on the morphological and ecological changes that characterize this period, metamorphosis is also the time of physiological modifications. Among them, changes in the capacity to osmoregulate at metamorphosis have been reported in several teleost species. Similar metamorphic modifications of osmoregulation are also known in other aquatic groups such as crustaceans. Later in the development of zebrafish, we found that blood osmolality did not change and stayed around 300 mOsm·kg⁻¹ in the tested stages, juvenile-adults 2 to 3 months of age and 2-year-old adults. These ontogenetic changes in the capacity to osmoregulate are related to the development of ionocytes in several organs. After hatching at 2–3 dpf, these Na⁺/K⁺-ATPase-rich cells are first found on the skin of the larva, particularly on the yolk sac and they appear on the gills. Also from 2–3 dpf, the pronephros is formed and appears functional with evidence of ultrafiltration in glomeruli and abundant Na⁺/K⁺-ATPase in tubule ionocytes. Along active ion uptake from fresh water in the skin ionocytes that compensates diffusive ion loss, the pronephros most probably participates in osmoregulation by producing dilute urine that eliminates excess water while limiting ion loss.

In the European sea bass Dicentrarchus labrax, increased blood osmolality during development has been correlated to an increase in the number of ionocytes. In the zebrafish, large numbers of ionocytes also begin to occur on the gills around 5–7 dpf. Between this time and 14 dpf, the osmoregulatory function through ion uptake from fresh water appears to shift from the skin to the gills.

On the gills, at least five types of ionocytes have been identified expressing different transporter enzymes (such as Na⁺/K⁺-ATPase and V-H⁺-ATPase) and several ion channels: they are involved in Na⁺ uptake/H⁺ secretion/NH₄⁺ excretion, Ca⁺⁺ uptake, Na⁺/Cl⁻ uptake, K⁺ secretion, and Cl⁻ uptake/HCO₃⁻ secretion. In particular, regarding the uptake of one of the major osmoregulator ions from fresh water, physiological evidence points to the role of Na⁺/H⁺ exchanger-, of H⁺-ATPase/ENaC-, and of NCC-mediated Na⁺ uptake. In addition, several hormones, among them prolactin and cortisol, have been shown to regulate ion transport through specific receptors.

The timing of occurrence of these cells and of the different transporters, and of the release of the hormones involved in ion transport is still only partly known, and future research should be directed at their ontogeny. We thus hypothesize that the increase in body fluids/blood osmolality observed in the present study between 5-day-old larvae and 3-week-old larvae originates from a higher ion uptake efficiency of the gills compared with the skin, and also perhaps from an improved function of the kidney. The structure and functions of zebrafish ionocytes have been mainly studied during early development, in embryos and early larvae. As the osmoregulatory performance increases at metamorphosis, it would be worthwhile to explore their changes during the metamorphic transition.

Slight differences in the surrounding medium osmolality have been shown to induce alteration of the structure of zebrafish organs such as eye lenses. Blood osmolality has thus to be taken into account for histological or experimental work as cell and tissue culture. We have found that blood osmolality increases by ~60 mOsm·kg⁻¹ through different developmental steps from early larvae to adults. These values can be used to adjust the osmolality of fixative, cell and tissue culture media, etc., according to the studied postembryonic stage.

In conclusion, during the postembryonic development of D. rerio, blood osmolality is close to 240 mOsm·kg⁻¹ in early larvae. It progressively increases to ~270 mOsm·kg⁻¹ during the larval development before reaching ~300 mOsm·kg⁻¹ after metamorphosis in juveniles, and later in adults. These ontogenetic changes in blood osmolality illustrate the physiological changes in osmoregulation associated with organ development in early larvae and later with metamorphosis. They open to possible further studies on the cellular (ionocytes) and molecular modifications associated with the changes in osmolality at metamorphosis. Finally, these values are of practical interest for adjusting the osmolality of fixatives and cell and tissue culture media for research on the zebrafish model.

Authors’ Contributions

G.C.: conceptualization (lead); data curation (equal); formal analysis (lead); funding acquisition (supporting); investigation (lead); methodology (lead); resources (equal); supervision (lead); visualization (supporting); writing original, draft (lead); and writing review editing (lead). M.N.-C.: data curation (lead); formal analysis (equal); funding acquisition (supporting); investigation (supporting); visualization (supporting); writing original, draft (equal); and writing review editing (supporting). G.L.: conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (supporting); investigation (supporting); methodology (equal); resources (supporting); visualization (lead); and writing review editing (supporting). T.A.: conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (supporting); investigation (equal); methodology (equal); resources (supporting); visualization (supporting); and writing original, draft (equal); and writing review editing (equal).

Acknowledgment

The authors thank Catherine Gonzalez and Stephane Castel for taking care of the fish facility.

Disclosure Statement

No competing financial interests exist.

Funding Information

This work was supported by a grant from the European Community’s H2020 Program [Marie-Curie Innovative Training Network ImageInLife: Grant Agreement no 721537]. Funding sources had no role in the writing of the article or the decision to submit it for publication.

Supplementary Material

Supplementary Table S1

References

1. Spence R, Fatema MK, Reichard M, et al. The distribution and habitat preferences of the zebrafish in Bangladesh. J Fish Biol 2006;69:1435–1448.
2. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Dev Dynam 1995;203:253–310.
3. Parichy DM, Elizondo MR, Mills MG, Gordon TN, Engesser RE. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. Dev Dyn 2009;238:2975–3015.
4. Lawrence C. The husbandry of zebrafish (Danio rerio): a review. Aquaculture 2007;269:1–20.
5. Sawant MS, Zhan S, Li L. Effect of salinity on development of zebrafish, Brachydanio rerio. Curr Sci 2001;81:1347–1350.
6. Hoshijima K, Hirose S. Expression of endocrine genes in zebrafish larvae in response to environmental salinity. J Endo 2007;193:481–491.
7. Boisen AMZ, Amstrup J, Novak I, Grosell M. Sodium and chloride transport in soft water and hard water acclimated zebrafish (Danio rerio). Biochem Bioph Acta 2003;1618:207–218.
8. Ullano E, Cataldi M, Carella F, Migliaccio O, Iacarino D, Agnisola C. Effects of acute changes in salinity and temperature on routine metabolism and nitrogen excretion in gambusia (Gambusia affinis) and zebrafish (Danio rerio). Comp Biochem Physiol A Mol Integr Physiol 2010;157:283–290.
9. Evans DH, Claiborne JB: Osmotic and ionic regulation in fishes. In: Osmotic and Iton regulation—Cells and Animals. Evans DH (ed), pp. 295–366. CRC Press, Boca Raton, FL, 2009.
10. Jonz MG, Nurse CA. Epithelial mitochondria-rich cells and associated innervation in adult and developing zebrafish. J Comp Neurol 2006;497:817–832.
11. Horng JL, Lin LY, Huang CJ, Katoh F, Kaneko T, Hwang PP. Knockdown of V-ATPase subunit A (atp6v1a) impairs acid secretion and ion balance in zebrafish (Danio rerio). Am J Physiol Regul Integr Comp Physiol 2007;292:R2068–R2076.
12. Hsiao CD, You MS, Guh YJ, Ma M, Jiang YJ, Hwang PP. A positive regulatory loop between foxi3a and foxi3b is essential for specification and differentiation of zebrafish epidermal ionocytes. PLoS One 2007;3:e302:1–17.
13. Janicke M, Carney TJ, Hammerschmidt M, Foxi3 transcription factors and Notch signaling control the formation of skin ionocytes from epidermal precursors of the zebrafish embryo. Dev Biol 2007;307:258–271.
14. Esaki M, Hoshijima K, Nakamura N, et al. Mechanism of development of ionocytes rich in vacuolar-type H+-ATPase in the skin of zebrafish larvae. Dev Biol 2009;329:116–129.
15. Lin LY, Horng JL, Kunkel JG, Hwang PP. Proton pump-rich cell secretes acid in skin of zebrafish larvae. Am J Physiol Cell Physiol 2006;290:C371–C378.
16. Barasona MI, Molina A, Blanco A, Ayala N, Moyano R. Assessment of the effects of bisphenol-A as a disruptor on ionic regulation in Danio rerio zebrafish through a study of their chloride and prolactin cells. Acta Adriat 2017;58:105–116.
17. Hwang PP. Review. Ion uptake and acid secretion in zebrafish (Danio rerio). J Exp Biol 2009;212:1745–1752.
18. Hwang PP, Chou MY. Zebrafish as an animal model to study ion homeostasis. Pflugers Arch Eur J Physiol 2013;465:1233–1247.
19. Guh YJ, Lin CH, Hwang PP. Osmoregulation in zebrafish: ion transport mechanisms and functional regulation. EXCLI J 2015;14:627–659.
20. Craig PM, Wood CM, McCleland GB. Gill membrane remodeling with soft-water acclimation in zebrafish (Danio rerio). Physiol Genom 2007;30:56–60.
21. Kozlowski TM, Jönsson M, Ek F, Olsson R, Kröger RHH. 2018. Osmotic concentration of zebrafish (Danio rerio) body fluids is lower in larvae than in adults. Zebrafish 2018;15:9–14.
22. Parry G. Osmotic adaptation in fishes. Biol Rev Camb Philos Soc 1966;41:392–444.
23. Rombough P, Gills are needed for ionoregulation before they are needed for O2 uptake in developing zebrafish, Danio rerio. J Exp Biol 2002;205:1787–1794.
24. Hill AJ, Bello SM, Prasch AL, Peterson RE, Heideman W. Water permeability and TCDD-induced edema in zebrafish early-life stages. Toxicol Sci 2004;78:78–87.
25. Perez-Camps M, Garcia-Ximenez F. Osmolarity and composition of cell culture media affect further development and survival in zebrafish embryos. Animal 2008;2:595–599.
26. Cardona-Costa J, Francisco-Simao M, Perez-Camps M, Garcia-Ximenez F. Micromanipulation medium osmolarity compromises zebrafish (Danio rerio) embryo and cell survival in chiameiram experiments. Zygo 2010;18:155–158.
27. Bone Q, Moore RH. Osmoregulation and ion balance. In: Biology of Fishes, 3rd ed. Owen E (ed), pp. 161–187. Taylor & Francis, New York, Abingdon, 2013.
28. Alderdice DF. Osmotic and ionic regulation in teleost eggs and larvae. In: The Physiology of Developing Fish: Eggs and Larvae. Fish Physiology, 11A. Hoar WS and Randall DJ (eds), pp.163–242, Academic Press, London, 1998.
29. Varsamos S, Connes R, Diaz JP, Barnabé G, Charmantier G. Ontogeny of osmoregulation in the European sea bass Dicentrarchus labrax. L. Mar Biol 2001;138:909–915.
30. Varsamos S, Nebel C, Charmantier G. Ontogeny of osmoregulation in post-embryonic fish: a review. Comp Biochem Physiol A Mol Integr Physiol 2005;141:401–429.
31. Charmantier G. Ontogeny of osmoregulation in crustaceans: a review. Invertebr Reprod Dev 1998;33:177–190.
32. Holmes WN, Donaldson EW. The body compartments and the distribution of electrolytes. In: Excretion, ionic regulation, and metabolism. Fish Physiology, 1. Hoar WS and Randall DJ (eds), pp. 1–89, Academic Press, New York, San Francisco, London, 1969.
33. Hickman CP Jr., Trump BF. The kidney. In: Excretion, ionic regulation, and metabolism. Fish Physiology, 1. Hoar WS and Randall DJ (eds), pp. 91–239, Academic Press, New York, San Francisco, London, 1969.
34. McMenamin SK, Parichy DM. Metamorphosis in teleosts. Curr Top Dev Biol 2013;103:127–165.
35. Charmantier G, Charmantier-Daures M, Aiken DE. Metamorphosis in the lobster Homarus (Crustacea Decapoda): a review. J Crust Biol 1991;11:481–495.
36. Pan TC, Liao BK, Huang CJ, Lin LY, Hwang PP. Epithelial Ca2+ channel expression and Ca2+ uptake in developing zebrafish. Am J Physiol Regul Integr Comp Physiol 2005;289:1202–1211.
37. Drummond IA, Majumdar A, Hentschel H, et al. Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. Development 1998;125:4655–4667.
38. Varsamos S, Diaz JP, Charmantier G, et al. Location and morphology of chloride cells during the post-embryonic development of the European sea bass, Dicentrarchus labrax. Anat Embryol 2002;205:203–213.

Address correspondence to:
Guy Charmantier, PhD
CNRS, Ifremer, IRD, UM, Marbec University of Montpellier
Pl. E. Bataillon
Montpellier 34095
France

E-mail: guy.charmantier@umontpellier.fr