Review

The effect of vitamin A on flatfish development and skeletogenesis: A review

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ABSTRACT

Flatfish can provide an excellent model to study developmental alterations of cartilage and bone tissues in response to vitamin A (VA), and therefore, with respect to the underlying morphogenesis, cell differentiation and proliferation processes, and gene expression regulation. Bone and cartilage development and homeostasis at different life stages (embryonic, larval, juvenile or adult stages) are primarily regulated by retinoic acid, the active metabolite of VA. Since fish are not able to synthesize VA, they have to obtain it from the diet. Thus, dietary VA imbalance may modify VA homeostasis, resulting in a disruption of gene expression, and cell proliferation and differentiation of skeletal tissues (bone and cartilage), leading to the appearance of skeletal deformities. These skeletal deformities are one of the most important factors that affect production efficiency and quality of flatfish aquaculture. However, different flatfish species present different developmental timing in skeletogenesis and consequently, different species may present different VA requirements. In the present work, special attention has been made to different experimental approaches to evaluate the effects of this morphogenetic nutrient on early morphogenesis (bath exposure vs. dietary dose–response nutritional essays), as well as the different retinoid forms in which VA was administered to larvae. We review the current state of knowledge on the effects of dietary VA content in flatfish development with an emphasis on the skeletal deformities induced by dietary VA imbalances.

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1. Introduction

Since 1970, interest on flatfish aquaculture has grown as the overexploitation of natural resources and environmental degradation have caused depletion of fisheries of several of these species, such as Japanese flounder (*Paralichthys olivaceus*), summer flounder (*P. dentatus*), turbot (*Psetta maxima*) and Atlantic halibut (*Hippoglossus hippoglossus*) (Bengtson, 1999). However, within marine fish aquaculture, world flatfish farming is considered as a minor activity. In 2008, it only represented around 10% of the total production of marine fish species (1,511,087 tonnes; FAO, 2010). However, driven by the high market prices in many Asian and European countries, where they are a cultural source of food, flatfish production has increased from 26,310 to 148,807 tonnes between 2000 and 2008, which represents an increase of 565% in production and 50% in value (FAO, 2010).

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During the last decade, flatfish production has been hampered by an array of biological and physiological difficulties (Bengtson, 1999) such as: pigmentary disorders (Bolker and Hill, 2000; Gavaia et al., 2002; Hamre et al., 2007); high mortality rate during the weaning feeding phase (Zarza et al., 2003); low quality and discontinuous spawning (Howell et al., 2009); and/or high incidence of skeletal deformities (*e.g.* Takeuchi et al., 1998; Gavaia et al., 2002; Fernández et al., 2009, among others). Skeletal deformities reduce the economic efficiency of the fish production process (Divanach et al., 1996). Hatcheries that provide juveniles grade out malformed fish while, those farms that grow out the fish to market size have either to downgrade the product to a lower value or discard the deformed fish entirely (Michie, 2001). The extent of losses at either point is different, dependent on the species and the husbandry practices followed. In whatever circumstance, these losses are substantial, in terms of both productivity and profitability. Hatchery and nursery productions are mostly affected, since the majority of skeletal structures develop during the larval and juvenile stages, in which organogenesis and morphogenesis take place. The development of skeletal disorders has been linked to a poorly understood relationship between environmental and biotic factors (reviewed in Lall and Lewis-McCrea, 2007). Among these, larval nutrition at first feeding is one of the key parameters affecting skeletogenesis during early development. Several works have shown that suboptimal levels and different forms of nutrients supplied in the diet are responsible for the appearance of skeletal deformities (Cahu et al., 2003; Lall and Lewis-McCrea, 2007). Both environmental and nutritional requirements closely correlate to the larval developmental stage and the skeleton ontogeny and anatomy of each fish species. Consequently, understanding the environmental needs and nutritional requirements of fish larvae is essential for improving husbandry and rearing practices, and reducing the incidence of skeletal disorders and their associated economical costs. However, while mammalian nutritional effects on bone development and remodelling have been widely studied, in fish these types of studies are limited and depend on the nutrient and fish species under consideration. Research on compound diet composition for marine finfish larvae and enriching emulsions for live preys has revealed that several nutrients, particularly minerals, vitamins, and lipids are critical for normal fish morphogenesis and skeletogenesis (Lall and Lewis-McCrea, 2007).

Retinoids, also known as Vitamin A (VA), are a group of morphogenetic nutrients that include all compounds that possess the same biological activity as retinol, and since fish are not able to synthesize VA, they have to obtain it from the diet at the optimum level, but also in the proper chemical form. In general, retinoids and their precursors (carotenoids) are absorbed from the diet by means of a complex metabolism, which includes a high number of transport proteins and enzymes, that at the same time, tightly control retinoid absorption, transport, accumulation and conversion to active forms. There are two primary active forms: (i) retinal, used as the chromophore of rhodopsin in the eye (Pepe, 1999); and (ii) retinoic acid (RA), the main active metabolite of VA that represents the other non-visual functions of VA (Ross et al., 2000). RA binds to nuclear RA receptors (retinoic acid receptors and retinoid X receptors, RARs and RXRs, respectively) and through them, regulates gene expression, cellular differentiation and proliferation processes (Ross et al., 2000), determining body pattern, growth, and the development of skeletal structures, as well as being an important element in other developing systems such as the nervous and immune systems (Fig. 1).

The impact of dietary VA on flatfish development and aquaculture will depend on the following factors: the chemical form and dose of VA in the diet, the duration of this supply, the fish developmental stage, and the particular ontogeny of the species. Therefore, dietary VA requirements need to be fine-tuned for each flatfish species, although they share similar skeletal structures and a common developmental process from a symmetrical to an asymmetrical skeleton and shape (Watanabe et al., 2008). To date, different approaches have been used to understand how VA affects different aspects of aquaculture production: from adult fish (reproductive performance) to larvae and juveniles; although special emphasis has been placed on flatfish skeletogenesis and pigmentation disorders, with larvae at first feeding being the developmental stage most studied. This article reviews all the research conducted studying dietary VA effects on skeletogenesis of different flatfish species.

2. Flatfish skeletal development and metamorphosis

Generally, marine fish larvae hatch much earlier than other vertebrates, and their development continues several days/months afterwards, representing a good biological model for studying the process of skeletogenesis (Haga et al., 2002a). The formation of the skeleton in Teleosts is quite different in their spatiotemporal sequences from those of higher vertebrates. In particular, fish larvae undergo morphogenesis and skeletogenesis during their early ontogeny, developing all the organs and systems that will lead the larva into a juvenile, which in the case of flatfishes becomes more complicated due to their metamorphosis and bottom settlement. A thorough description of the metamorphosis process and its endocrine regulation is outside the scope of this review, but readers can consult recent studies from Geffen et al. (2007), Power et al. (2008) and Klaren et al. (2008).

In brief, metamorphosis is a post-embryonic process, which involves profound morphological, biochemical and physiological transformations in order to adapt to a new habitat. In flatfish, it is an endocrine driven process in which thyroid hormones play a central role (Power et al., 2008), where fishes have a dramatic transformation from a bilateral symmetrical to an asymmetrical anatomy. Studies in zebrafish (*Danio rerio*) demonstrate that the nodal-lefty-pitx2 (NLP) pathway directs organ sidedness by promoting the expression of these genes on the left side of their embryonic primordia that in flounders occur irrespective of whether the species exhibits sinistral or dextral asymmetry (Hashimoto et al., 2007). *Pitx2* is the final left–right determinant of the NLP pathway (Suzuki et al., 2009), as loss of *pitx2* re-expression induces randomization of eye-sidedness, manifesting as normal, reversed or bilateral symmetry, with the laterality of the structural asymmetry of the habenula entirely inverted in reversed flounders compared with normal ones. Perhaps the most striking developmental feature in flatfishes (presumably evolved as an adaptation to their benthic existence) is the eye-sidedness. The migration of one eye to the opposite side of the head requires extensive rearrangement and remodelling of tissues, which occur at the level of individual morphological elements, rather than as a global distortion of the entire chondrocranium (Martinez and Bolker, 2003). During metamorphosis, the fish skeleton experiences the following major morphological changes: (i) a 90° rotation of the cranial and visceral skeleton; (ii) eye migration to the ocular upper side resulting in neurocranium and splachnocranium reorganization; (iii) development of the pseudomesial bone during eye migration (Okada et al., 2003; Sæle et al., 2006a); and (iv) remodelling of the pectoral fin (Schreiber, 2006). The timing of this process varies among flatfish species and seems to depend on the different water temperatures at which they live. Osse and Van den Boogaart (1997) proposed two general patterns of metamorphosis: (a) plaice-like, where metamorphosis occurs at larger larval sizes (as in European plaice *Pleuronectes platessa* and Atlantic halibut *Hippoglossus hippoglossus*); and (b) sole-like, in which metamorphosis takes place at smaller larval sizes and has a short duration (as in Senegalese sole *Solea senegalensis*). For example, while metamorphosis in Atlantic halibut starts at 80–90 days post hatch (dph) or 1035 °C day and finishes 45 days after (at 1552 °C day), in Senegalese sole this begins at 10 dph (170 °C day) and finishes approximately 10 days later (340 °C day), depending on the rearing temperature (Fernández et al., 2009; Gavaia et al., 2002). Furthermore, even flatfish species within the same genera