Aquaculture 488 (2018) 189–198

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

# Understanding the micro-elemental nutrition in the larval stage of marine fish: A multi-elemental stoichiometry approach

Jian Wang, Wen-Xiong Wang*

*Division of Life Science, Hong Kong University of Science and Technology (HKUST), Clearwater Bay, Kowloon, Hong Kong*

ARTICLE INFO

*Keywords:*
Stoichiometry
Micro-element
Nutrition
Larval fish

ABSTRACT

In the present study, we for the first time systematically investigated the multi-elemental stoichiometric changes in three marine fish larvae (marine medaka, gilthead seabream and golden pompano). These larvae were raised up to 4-week post-hatching using live feeds (i.e., rotifer and *Artemia*), and micro-elemental (i.e., Zn, Cu, Fe, Mn, Co and Se) requirements in the brood stock and early larval stage were evaluated from the very basic elemental levels. Different micro-elements levels were found in the newly hatched larvae, especially in pompano with a comparatively high Zn (210 mg/kg dry wt.) and Se (3.4 mg/kg) concentration. Diverse micro-elemental concentrations and changes were also observed in the three fish during development, especially around the mix-feeding stage. By using micro-element/P ratios, we found that seabream and pompano required more micro-elements than medaka. Furthermore, literature available data on micro-element/P ratios in some other marine fish larvae (e.g., Atlantic cod, red seabream and amberjack) and different live feeds (e.g., rotifer, *Artemia* and copepods) were calculated. By comparing these ratios, the importance of copepods in micro-elements supply to marine fish larvae was demonstrated. Due to the potential high demand of fish at the very early life stage, Zn and Se were likely to be limited when supplied with rotifers, while Fe, Cu, Co and Mn should generally meet the fish requirements. Potential shortage of Fe, Mn and Co from non-enriched *Artemia* may occur due to the increasing demands at late larval stage, which could be relieved by co-feeding with rotifer or enrichment. This study shows the diverse micro-element concentrations and changes during early fish ontogeny. More future multi-elemental stoichiometry studies are required to have a deep understanding of the micro-elemental requirements in the early life stage of fish.

## 1. Introduction

With increasing seafood demand, aquaculture has become the fastest growing food industry at the annual rate of ca. 9% (Allan and Burnell, 2013). However, supply of the healthy fingerlings is still the bottleneck for the continuing growth of this industry. Factors such as brood stock health, water quality, pathogeny and nutrition all affect the survival of larval fish (Allan and Burnell, 2013; Hamre et al., 2013). Many studies have addressed the feeding behavior, digestive physiology, and major nutrient requirements (Holt, 2011). A typical example was the production of Gilthead seabream. In the 1960s to 1970s, successful reproduction of seabream was achieved, but with a very low larval survival (ca. 1%). Advancement of larval fish nutrition then rapidly promoted this industry with the global annual production reaching 158,389 tons in 2014 (FAO, 2014). Besides, live feeds (e.g., rotifer and *Artemia*) enrichment became a general practice to significantly improve the performances (e.g., growth and survivability) of larval fish (Dhont et al., 2013; Øie et al., 2011). For example, rotifer enriched with docosahexaenoic acid (DHA) showed a positive effect on the survival and growth of Atlantic cod larvae (Park et al., 2006), and a combination of DHA and arachidonic acid enrichment increased the resistance of seabream larvae to stress (e.g., grading) (Koven et al., 2001).

There are still concerns on how to enhance the brood stock nutrition in order to generate nutritionally sufficient embryos and whether the added food can meet the nutritional requirements of the larval fish. Elements in the embryo directly reflect the maternal investments, and sufficient elemental provision of the fish embryos ensures the development of free swimming larvae since most of the larvae feed freely before the arrival of “point of no-return” several days later (McGurk, 1984). Insufficient elemental provision leads to low hatchability of the fish embryos. Previously, Takeuchi el al. (1981) observed that rainbow trout fed diets with limited micro-elements (i.e., Fe, Mn and Zn) produced eggs with low micro-element concentrations and low hatchability. Thompson et al. (2012) also observed positive correlation between Zn concentration and egg viability in squirrelfish. Therefore, it is

* Corresponding author.
*E-mail address:* wwang@ust.hk (W.-X. Wang).

https://doi.org/10.1016/j.aquaculture.2017.12.036
Received 13 July 2017; Received in revised form 18 December 2017; Accepted 21 December 2017

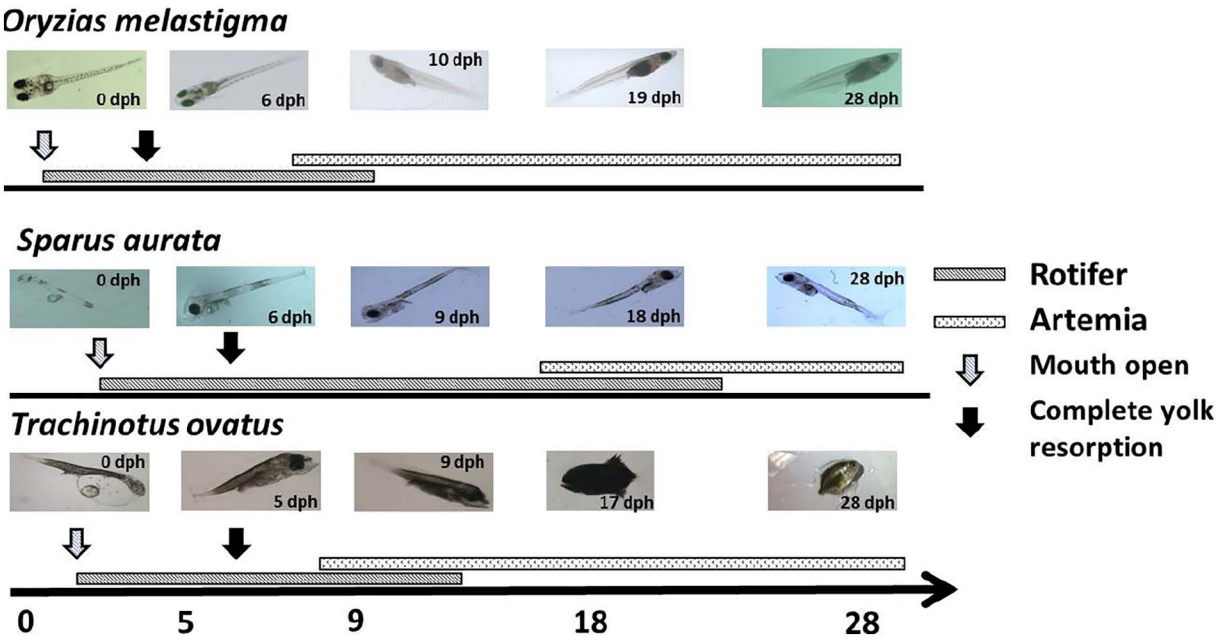


**Fig. 1.** Developing events and feeding regimes for marine medaka (*Oryzias melastigma*), Gilthead seabream (*Sparus aurata*) and golden pompano (*Trachinotus ovatus*).

crucial to determine the sufficient micro-elemental inclusion levels in the embryo, especially identifying those species with extraordinary high micro-element requirements.

Unlike juvenile and adult stages, the larval stage is characterized by rapid metamorphosis (Miller and Kendall, 2009), and whether a "constant elemental concentration" can stand for the whole life stage requirement is questionable. The larval fish keep switching its preys as they grow in the wild environment and these preys are not homogenous in nutritional composition as well, such as the micro-elements (Table S1). Compared to macro-nutrients (e.g., free amino acids, lipids and carbohydrates), the micro-elemental requirements have not been adequately quantified in marine larvae fish (Moren et al., 2011). Previously, dynamic Zn and Fe requirements (based on flux) were found in the growth of medaka (Wang and Wang, 2015, 2016). In practice, rotifer, copepod and *Artemia* are the three live feeds mostly applied for different fish larvae, regardless of the differential ontogeny of each fish species (Dhont et al., 2013). Hence, it is necessary to understand the dynamic nutritional requirements of each fish in order to modify the nutrient composition in the live feeds.

Elemental stoichiometry depicts the relative quantities of different elements in an organism (Sterner and Elser, 2002). By studying the elemental stoichiometry in different organisms, it is possible to diagnose the regulation of nutrients in biota and study the elemental cycles in ecosystems (Sterner and Elser, 2002). One of the basic premises in ecological stoichiometry is homeostasis, in which the elemental composition of an individual is assumed constant throughout its lifespan. Sterner and George (2000) found less variability of elements (i.e., C, N and P) in different cyprinid fish than in its gut content, supporting a homeostatic model of nutrient flux. Homeostatic regulation was also ubiquitously found in essential micro-elements (e.g., Cu, Fe and Zn) for different fish species (Wood, 2011), which resulted a relatively constant micro-element concentration. However, most of these conclusions were based on the adult fish, whereas the rapid growing and developing larval stage has been seldom included. A recent study in two different minnow fish found significant variable elemental (i.e., C, N, P and Ca) composition among development stages, and the homeostatic assumption was not applicable to these early fast growing larvae (Boros et al., 2015). Thus, understanding the basic elemental stoichiometry in larval fish would provide fundamental information to the nutritional requirement. Specifically, it is possible to directly quantify the micro-elements (e.g., Cu, Fe and Zn) requirement from the elemental stoichiometry.

To address these questions, we investigated the elemental stoichiometry in three marine fish larval, namely marine medaka (*Oryzias melastigma*), Gilthead seabream (*Sparus aurata*) and Golden pompano (*Trachinotus ovatus*). Both macro- (i.e., C, N, P and Ca) and micro- (i.e., Cu, Fe, Zn, Co, Mn, and Se) elements were measured over the first four weeks of development. Micro-element concentrations and micro-element/P (M/P) ratios were used to evaluate the nutritional requirement in brood stock and larval fish, and the corresponding sufficiency of typical used live feeds (i.e., rotifer, *Artemia* and copepod).

## 2. Materials and methods

### 2.1. Organisms

Marine medaka brood stocks were reared in 90 L glass aquarium using the natural seawater (Clearwater Bay, Hong Kong). Newly hatched *Artemia nauplii* were delivered to the brood stocks at a total daily amount of 5% body wet wt. in three times. Medaka embryos were collected by siphon and incubated at 25 °C until hatching. For seabream and pompano, fertilized eggs from the same batch of brood were obtained from a nearby fish farm (Nan'ao, Guangdong Province, China) on Jan 3rd, 2016 and May 5th, 2016, respectively. The brood stocks (i.e., seabream and pompano) were fed cuttlefish and krill meals during vitellogenesis periods. Eggs were transported to the laboratory within 3 h and incubated in 500 L glass fiber aquarium. We chose these species because they were easily reared under the laboratory conditions and different in ontogeny. Therefore, it was possible to explore the nutrient requirements of larval fish with variable ontogeny strategies under the laboratory conditions (Fig. 1).

### 2.2. Larval fish culturing

Medaka egg hatched after 10 d-incubation (salinity 33 ppt, temperature 25 ± 1 °C). The newly hatched medaka were gently transferred into 20 L glass aquarium (with 15 L seawater) at a density of 30 ind./L in natural seawater (salinity 33 ppt, temperature 25 ± 1 °C, pH = 8.0 ± 0.3 and DO > 6 mg/L). Illumination (12 h Light: 12 h Dark) and gentle aeration were provided right after hatching. Under