Chapter 3
Weaning Regimes for Golden Pompano *Trachinotus ovatus* Larvae

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Abstract This chapter covers the digestive ontogeny of *T. ovatus* from hatch to 32-day post-hatch (DPH). The development of the digestive system in *T. ovatus* can be divided into three stages: stage I starting from hatching and ending at the onset of exogenous feeding (3 DPH), stage II starting from first feeding and ending at the formation of gastric glands in the fish stomach (15 DPH), and stage III starting from the appearance of gastric glands and continuing onward. The specific activities of lipase, trypsin, and amylase in fish increased sharply from the exogenous feeding to 5–7 DPH. The pepsin activity was detected on 15 DPH, and the specific activities increased with fish age. The dynamics of enzyme activity reflected the structural development in the fish digestive system. After the formation of gastric glands in the stomach, the enzyme activities became stable. Depending on the development of the digestive system, the larvae of *T. ovatus* can begin weaning at 15 DPH. This chapter updates the improved understanding of the ontogeny of *T. ovatus* during the larval phase and provides the protocol of feeding and weaning for this economically important fish in aquaculture.

Keywords Ontogenetic development · Digestive system · Enzyme activity · *Trachinotus ovatus*

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3.1 Introduction

Upon hatching, the digestive system of marine fish larvae is immature and undergoes major changes before fully having the function of food digestion (Cahu and Zambonino-Infante 2001; Hu et al. 2018; Ma et al. 2012). In spite of advances in larval fish culturing technology, most of marine fish hatcheries still depend on live feed such as rotifers *Brachionus* spp. and *Artemia* sp. as feed for early larvae of fish (Hamlin and Kling 2001; Sorgeloos et al. 2001; Hu et al. 2018). In practice, live feed supply is usually required beyond metamorphosis until larvae are weaned onto formulated diet (Alves et al. 2006; Ma et al. 2014; Cui et al. 2017). Nevertheless, the long-term use of live feed is costly and may lead to malnutrition due to unbalanced nutrition, and live feed does not meet the nutritional requirement for larval fish (Le Ruyet et al. 1993; Baskerville-Bridges and Kling 2000; Callan et al. 2003). Thus, the weaning of fish larvae at their early stage is essential.

Weaning with artificial feed instead of live feed is a gradual process during the larval stage. In aquaculture practice, most temperate marine fish, such as *Lutjanus erythopterus* and *Seriola lalandi*, usually begin weaning after metamorphosis (Cui et al. 2017; Ma et al. 2014). Marine fish can make weaning easier to manage and more effective if compound diet is introduced early (Baskerville-Bridges and Kling 2000; Hart and Purser 1996). It is likely to have a negative impact on the growth and survival of fish if artificial feed is introduced too early because larvae of fish have no ability to digest artificial feed (Andrade et al. 2012; Cahu and Zambonino-Infante 2001; Ma et al. 2014). The co-feeding protocol for fish larvae with both live and artificial feed has been developed in finfish hatchery to improve poor digestion of artificial feed at the early stage of development. The proportion of live feed is gradually reduced during the weaning stage, and the co-feeding strategy allows the fish larvae to receive artificial feed earlier in terms of nutrition (Rosenlund et al. 1997; Engrola et al. 2009b). The growth, survival, and the quality of marine fish species can be significantly improved by co-feeding such as *Sciaenops ocellatus* (Lazo et al. 2000), *Rhombosolea tapirina* (Hart and Purser 1996), and *Solea senegalensis* (Engrola et al. 2009a).

The response of fish to nutrient supply can be assessed by RNA/DNA ratio that has been used as an indicator for somatic tissue growth (Bailey et al. 1995; Buckley et al. 1999; Gwak et al. 2003). Since the amount of DNA per cell is almost constant, cross-species fish growth can be measured by the RNA/DNA ratio as a consistent measure (Pilar Olivar et al. 2009; Gwak and Tanaka 2001), whereas the quantity of RNA reflects the amount of protein synthesis in cells (Höök et al. 2008; Tanaka et al. 2008). Because the nutritional condition is related to feeding success and food supply (Tanaka et al. 2008), fish with an adequate nutrition supply may have a higher RNA/DNA ratio than malnourished fish (Boyd and Tucker 1992; Gronkjer et al. 1997).

Although fish can consume artificial diets in their early period of development, this does not guarantee the success of the artificial diet digestion and absorption, because the gut of fish may be filled with artificial diet and death (Cahu and

Zambonino-Infante 2001). Therefore, introduction time of artificial diet of fish larvae should be determined according to the development of digestive system (Cahu and Zambonino-Infante 2001). In some studies, the histological structure degrades when fish larvae are malnourished (Yufera et al. 1993; Chen et al. 2007). Midgut cells' height is a histological indicator of fish nutrient supply as evidenced by the early development of *Seriola lalandi* (Chen et al. 2007) and *Theragra chalcogramma* (Theilacker and Watanabe 1989).

The *Trachinotus ovatus* belongs to the Carangidae family and is extensive cultured in the Asia-Pacific region. We examined the development of the digestive system in previous studies; in particular, attention is paid to pepsin secretion and the appearance of gastric glands in the stomach (Ma et al. 2013). In this chapter, we discuss the suitable time of weaning for *T. ovatus*, aiming to improve fish survival and establish a cost-effective feed regime for the fish that are commercially important.

3.2 Weaning Scheme Design

On 12 DPH, the fish larvae were randomly divided into 12, 300-L experimental tanks (20 fish L^{-1}) for early weaning trial, which began on 13 DPH. The daily water exchange rate was 300% volume per day. Put an air stone in each tank to keep adequate dissolved oxygen and uniform the distribution of microalgae, rotifers, and *Artemia* nauplii. The weaning stage lasted for 10 days, including 5 days of co-feeding and 5 days of live feed combined with the introduction of artificial feed. Only feed the fish larvae with artificial diet after completion of weaning (Fig. 3.1).

Weaning treatments were composed of the same feeding protocol but began at four start time schemes after hatch: (1) start at 13 DPH and end at 22 DPH (W13), (2) start at 16 DPH and end at 25 DPH (W16), (3) start at 19 DPH and end at 28 DPH (W19), and (4) start at 22 DPH and end at 31 DPH (W22 as control); each group had

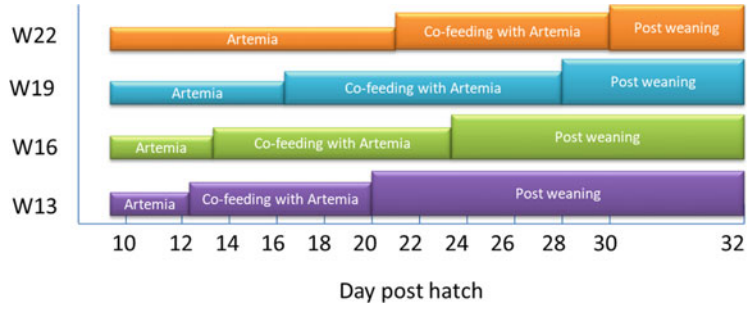

Fig. 3.1 Four co-feeding and weaning treatments to wean pompano *T. ovatus* larvae from 13 DPH to 32 DPH with separate commencing time by 3 days. W13: 13–22 DPH, W16: 16–25 DPH, W19: 19–26 DPH, and W22: 22–33 DPH (Ma et al. 2015)

three replicates. Each group has weaning interval for 3 days (i.e., 13, 16, 19, and 22 DPH, Fig. 3.1).

3.3 Growth, Survival, and Variation of RNA/DNA Ratios

The standard length (SL) of *T. ovatus* larvae on 1 DPH was 3.38 ± 0.17 mm (mean ± SD). When the weaning experiment was 13 DPH, the average SL of fish was 5.14 ± 0.44 mm. In the end, fish of W22 and W19 treatments increased significantly compared to fish in the W13 and W16 treatments ($P < 0.05$).

The specific growth rates of fish larvae of W13 and W16 treatments decreased significantly compared to fish in the W19 and W22 treatments, which were 7.32% day^{-1} and 7.82% day^{-1}, respectively ($P < 0.05$, Fig. 3.2). No significant difference of final survival rate of fish was found in W22 and W19 treatments, which were 85.17% and 86.5%, respectively ($P > 0.05$). No significant differences of final rates of fish were found in W13 and W16 treatments ($P > 0.05$), but it significantly decreased compared to fish in the W19 and W22 treatments ($P < 0.05$, Fig. 3.2).

Weaning time can significantly affect the growth of marine fish larvae (Curnow et al. 2006; Engrola et al. 2007). Inappropriate weaning time may lead to starvation of fish because of poor food digestion and absorption (Hu et al. 2018; Ma et al. 2012, 2014). There is no adequate nutrient supply during weaning period, and fish will use the energy stored in the body to maintain basic metabolism and to distribute less energy to growth, causing slow growth during the weaning period as reported in sand bass *Paralabrax maculatofasciatus* (Civera-Cerecedo et al. 2008) and *Senegalese sole* (Engrola et al. 2007). In *T. ovatus*, weaning time significantly affects the growth of fish larvae. The specific growth rates of fish of W19 and W22 groups were significantly higher than those of W13 and W16 groups. As we suggested in a previous study, *T. ovatus* is a fish with rapid development (Ma et al. 2013), and a functional digestive system appears at about 15 DPH. Higher fish growth rates were

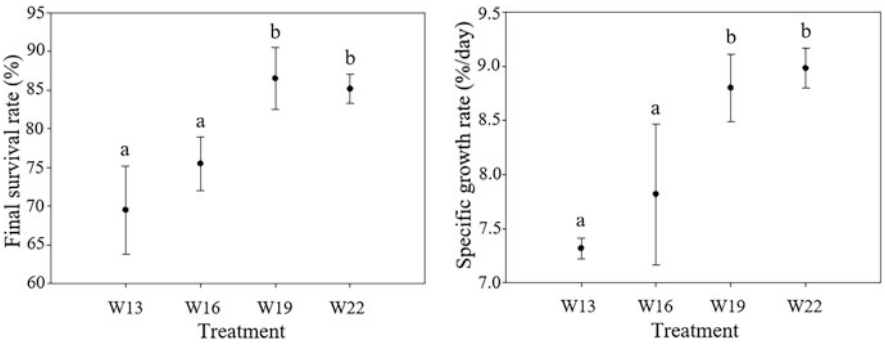

Fig. 3.2 Different weaning times, specific growth rate, and survival rate of *T. ovatus*. Different letters represent significant difference ($P < 0.05$). Abbreviations refer to Fig. 3.1 (Ma et al. 2015)

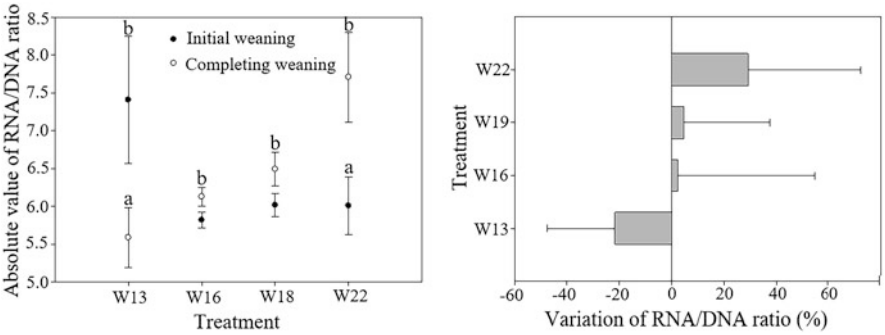

Fig. 3.3 The initial and final RNA/DNA ratios and variation of RNA/DNA ratios (VRD) in *T. ovatus* larvae during the weaning period. Different letters represent significant difference ($P < 0.05$). Abbreviations refer to Fig. 3.1 (Ma et al. 2015)

found in the W19 and W22 groups and may be associated with the function of the digestive system development.

During the ontogenetic development, the time of weaning regulated the RNA/DNA ratio ($P < 0.05$, Fig. 3.3). RNA/DNA ratio of fish of the W13 group at the end of weaning revealed a downregulating trend, and the ratio decreased from 7.4 to 5.6. A significant increase of the RNA/DNA ratios of fish after weaning was found in W16, W19, and W22 groups ($P < 0.05$, Fig. 3.3), and there was no significant difference in RNA/DNA ratios among these groups ($P > 0.05$, Fig. 3.3) though the mean variation of RNA/DNA ratios increased with the starting time of co-feeding and weaning (Ma et al. 2015).

The RNA/DNA ratios are used to understand growth pattern of many fish species during ontogenetic development (Pepin et al. 1999; Gwak et al. 2003; Höök et al. 2008). The RNA/DNA ratio of larval fish can be used to evaluate the fish nutritional condition because it is associated with food availability (Esteves et al. 2000; Diaz et al. 2011). The RNA content of larval fish such as turbot *Scophthalmus maximus* and herring *Clupea harengus* decreased when first feeding was not properly conducted (Clemmesen 1987). Previous studies have confirmed that the RNA/DNA ratio can be used to assess diet adequacy (Ben Khemis et al. 2000; Mendoza et al. 2008). In *T. ovatus*, weaning time can influence the change of the RNA/DNA ratio. When weaning was completed, the RNA/DNA ratio of fish of the W13 group indicated a reducing trend, which may suggest that weaning time has a negative impact on nutritional condition and growth of fish.

However, the positive variation of RNA/DNA ratios of fish was found in W16, W19, and W22 groups, which may indicate that fish adapt for artificial diet during weaning.

3.4 Height of Midgut Epithelial Cells

The epithelial cell height in the midgut of fish larvae is an excellent histological indicator to assess their nutritional condition (Gwak et al. 1999) because the form of enterocyte cells in the fish intestine can be changed by starvation (Domeneghini et al. 2002). The histological changes of fish larvae starvation vary with fish species and duration (Theilacker and Porter 1995; Gisbert et al. 2004; Ma et al. 2012). In larval yellowtail kingfish *Seriola lalandi*, the response of the midgut epithelium height to starvation occurred only before 33 DPH, and subsequently, there was no significant difference between normal fed and starved fish (Chen et al. 2007). In *T. ovatus*, the starting time of weaning significantly affected the intestinal epithelium, especially in the W13 treatment (Fig. 3.4). The height of epithelial cells of midgut of *T. ovatus* was decreased compared to the fish in the control group, from 15 DPH (W22), which is similar to the reports of Hamza et al. (2007) and Ostaszewska et al. (2005) where artificial diet is used to feed fish larvae. Starvation may cause a decrease of epithelial cell heights owing to incapability to digest artificial feed during the period of early weaning. However, the use of artificial diet results in low cell height because of intestinal epithelium damage (Hamza et al. 2007).

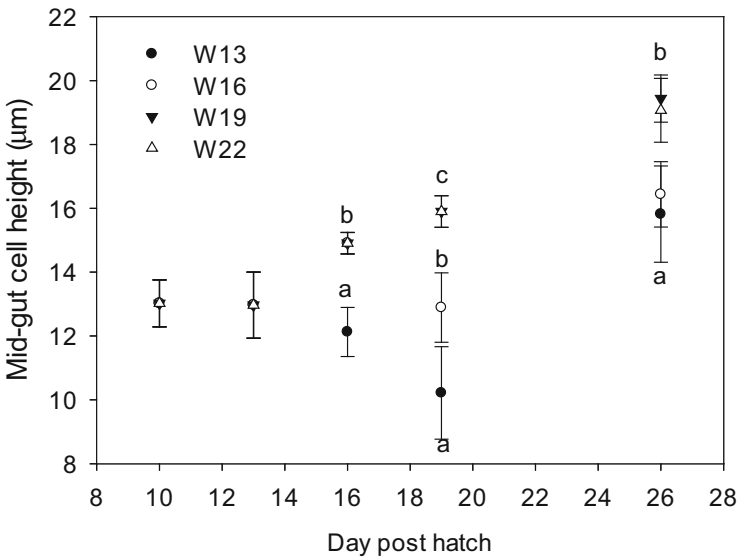

Fig. 3.4 The change of midgut cell heights of *T. ovatus* larvae in different weaning groups. Different letters represent significant difference ($P < 0.05$). Abbreviations refer to Fig. 3.1 (Ma et al. 2015)

3.5 Survival and Jaw Malformation

The co-feeding of live feed and artificial diet has been suggested as an effective program to improve the survival of larvae fish during weaning (Engrola et al. 2009a; Nhu et al. 2010; Clay et al. 2011). For example, whether artificial diet or *Artemia*, the alligator *Atractosteus spatula*'s survival rate is about 60% (Mendoza et al. 2008). However, the alligator's survival reached 95% when the alligator larvae were co-fed with 20% *Artemia* and 80% of artificial diet (Mendoza et al. 2002). In *T. ovatus*, co-feeding and weaning of fish larvae were successfully conducted. The survival rate of fish of W19 and W22 groups was higher (>85%) at the end of the experiment.

Nutrition is a significant factor affecting skeleton deformity (Cahu et al. 2003; Cobcroft et al. 2004; Sandel et al. 2010). During the period of co-feeding and early weaning, any inappropriate feeding protocol may lead to malnutrition of fish larvae.

The co-feeding time and early weaning time of marine fish larvae can affect the quality of fish such as skeletal malformation (Baskerville-Bridges and Kling 2000; Hamlin and Kling 2001). For example, compared with longer co-feeding and weaning time, shorter co-feeding and the time of weaning can improve spinal malformation incidence in southern flounder *Paralichthys lethostigma* (Faulk and Holt 2009). Similarly, during the weaning of pikeperch *Sander lucioperca*, a high malformation rate was found in the earlier weaning treatment (Kestemont et al. 2007). In this study, weaning time had no significant effects on jaw malformation ($P > 0.05$, Fig. 3.5). The jaw malformation rates were 15.54%, 11.78%, 14.00%, and 11.54% in the W13, W16, W19, and W22 treatments, respectively. The time of

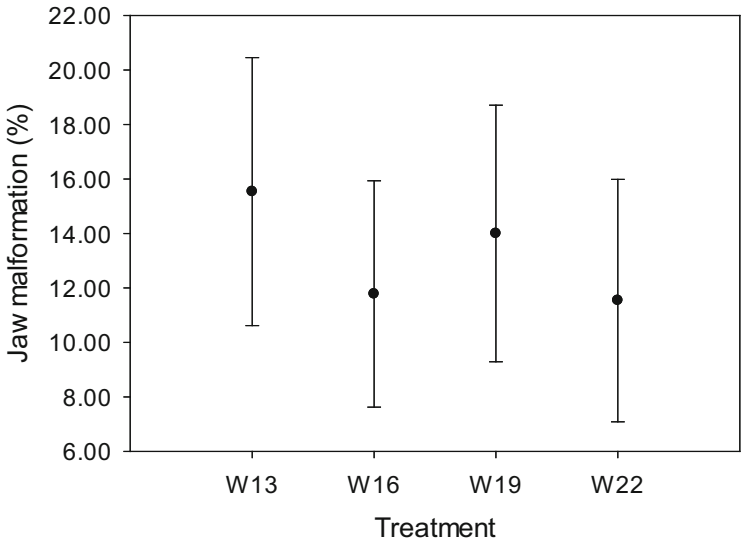

Fig. 3.5 The jaw malformation of *T. ovatus* larvae of different weaning groups. Different letters represent significant difference ($P < 0.05$). Abbreviations refer to Fig. 3.1

co-feeding and weaning cannot significantly affect the jaw malformation of larval *T. ovatus*. The existing evidence indicates that any of the current co-feeding and weaning regimes in this study can supply adequate nutrient to *T. ovatus* larvae during their development.

3.6 Conclusion

The *T. ovatus* larvae can be weaned from live feed to artificial diet after 16 DPH without affecting the fish growth and survival or increasing jaw deformity. However, the introduction of artificial diet before 16 DPH may affect the fish growth, survival, and nutritional condition. Although some *T. ovatus* larvae could be weaned on 13 DPH, we suggested the optimal time of weaning for *T. ovatus* larvae should be from 16 to 22 DPH.

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