International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Ingestion of Microencapsulated Diets by Japanese Flounder (*Paralichthys olivaceus*) Larvae

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Abstract: ***In order to study the feasibility of feeding young Japanese flounder (Paralichthys olivaceus) larvae with artificial diets, an experiment was designed to estimate the ingestion of a microencapsulated diet. Larvae from first feeding (3 days after hatching) were fed these microencapsulated diets. The experiment was carried out with new larvae every two days, as the larvae grew in a normal culture. Consumption was estimated by microscopic examination of the guts of the larvae fed microencapsulated diets. Results show that Japanese flounder larvae can ingest inert microcapsules right from first feeding, as long as the feed is available for a period longer than 10 minutes in the water column. The number of particles ingested and the size of the particles ingested increased with the age of the larvae. Another important conclusion is that the feed size must be 15-20 % of the mouth opening in order to be effectively ingested by the larvae.***

Keywords: microencapsulated diets, ingestion, Japanese flounder, larvae, first feeding

1. Introduction

Live foods such as rotifers, *Brachionus plicatilis,* and brine shrimp, *Artemia sp.* have been used for a long time as first foods for marine fish larvae. The failure to do this usually leads to high larval mortalities. Many studies have been carried out on the potential for replacement of live feeds in larval cultures [1]–[2]. Several authors have achieved the culture of fish larvae with microdiets [3]–[6]. However, all these experiments used co-feeding of live foods and artificial diets, somehow suggesting the difficulty of using an artificial diet as only feed for first feeding. The production of these live foods is difficult and expensive. In order to design a good larval diet, even before considering the nutrient requirements of the larvae, another basic factor must be considered: the feed must be ingested by the larvae. Only if the feed is ingested will the larvae have a chance of survival. Ingestion of live foods and artificial diets by freshwater fish has been measured by other researchers using gravimetric techniques [7]. In the present work, ingestion of microencapsulated diets by Japanese flounder larvae was estimated by microscopic examination of the guts of larvae offered microencapsulated diets at various ages.

2. Materials and Methods

2.1 Rearing method

Larvae of Japanese flounder 3 to 15 days after hatching were placed in 2 l beakers at a density of 50 larvae per litre, and were offered lipid walled microcapsules containing a red dye (Methyl Red, Kanto Chem. Co. Inc.) to facilitate its observation under the microscope. The larvae were sampled at regular intervals to check for the presence and number of particles in the gut, as well as the sizes of the particles ingested. The total number of feed particles in the gut were counted under the microscope, and the size of all visible particles were measured.

2.2 Diets

Lipid walled microcapsules were prepared following a method modified from Langdon [8]. The lipid wall was composed of tripalmitin, a lipid not easily digested by young marine fish larvae, in order to facilitate the identification of the unbroken particles under the microscope. These microencapsulated diets had no nutritional value, and were used only to estimate ingestion by the larvae on the day of the trial

3. Results

3.1 Ingestion of microencapsulated diets

The ingestion of microencapsulated diets by Japanese flounder larvae at different ages and sizes is shown on Table 1. Feed particles were observed in the gut of the larvae right from first feeding, confirming that fish larvae can ingest inert artificial diets without any attractant, as long as the diet is made available to the larvae. The ingestion of particles at this early stage was variable, observing fish containing from 0 to 40 particles in the gut. This fact may be due to the unsynchronized opening of the mouth by the young larvae. At this age (3 days after hatching) larvae proved to be slow feeders, since no particles were observed in the gut of the fish until more than 10 minutes after presenting the feed. This finding is very important in the design of an artificial diet for fish larvae. The buoyancy and water stability of the feed must be good enough to make the diet available to the larvae for a period long enough to be consumed. The number of particles ingested and the mean size of the particles ingested increased with the age of the larvae, even though all larvae were offered the same mix of microcapsule sizes.

Figure 1 shows 10 day old larvae with empty gut (A) and with the gut full of microcapsules (B). Larvae offered the microencapsulated diets ingested these particles eagerly, until the gut was virtually 100 % filled up.

Table 1: Ingestion of microencapsulated diets by Japanese flounder larvae at different ages and sizes.

age (days after hatching)	mouth opening (μm)	body length (mm)	number of particles per larvae	feed size (μm)
3	155	2.91	12.8	20.4
5	196	3.15	13.3	30.5
6	241	3.19	21.5	38.8
8	250	3.70	25.4	46.5
10	254	4.46	42.2	46.9
12	283	4.63	64.8	49.2
15	289	5.12	74.0	56.2

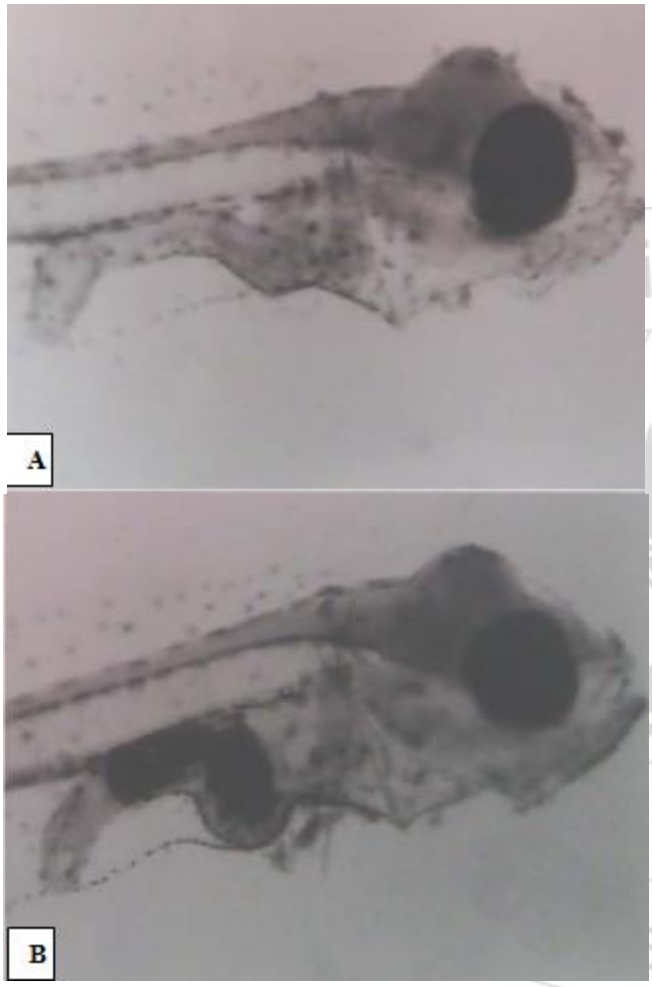

Figure 1: Ten day old Japanese flounder larvae with empty gut (A) and with gut full of ingested microcapsules (B)

4. Discussion

Microdiets have been used for a long time as weaning diets for marine fish larvae. In those studies, inert feeds were gradually introduced and live feeds were gradually replaced as the larvae grew [9]–[10]. The idea was that larvae were gradually used to ingest an inert food. This experiment shows that Japanese flounder larvae can ingest an inert food right from first feeding, without the need of co-feeding a live food. It also shows the importance of having a diet that is neutrally buoyant (i.e. stays on the water column, not sinking or floating to the surface) and is water stable. Most starter feeds are microparticles with negative buoyancy and limited water stability. Most of these feeds settle in the bottom of the tanks in a period much shorter than 10 minutes, thus becoming unavailable for the slow feeding young larvae. The use of these particulate diets may be a good choice for older, faster feeding larvae and juveniles, but it proves a poor choice for first feeding.

In our experiment, all larvae ingested particles of sizes much smaller than the corresponding mouth opening, suggesting that even though the larvae could swallow bigger particles, these particles could not enter the pharynx and were expulsed back to the water. This fact was confirmed by visual observations of the larvae in the culture vessels. This finding is very important for practical purposes. It was common practice to estimate the ideal particle size according to the mouth opening, but our results show that larvae selectively ingest particles of sizes only 15-20 % of the mouth opening. Another interesting result is the fact that larvae ingested an inert feed without any attractant, and virtually flavorless. In previous research we found that the ingestion of particulate feeds by larger Japanese flounder larvae may be reduced if the diet contains high levels of ingredients that are not very palatable [2]. The present results clearly indicate that attractants are not needed when using microencapsulated diets, opening up a lot of new possibilities regarding raw material uses.

5. Conclusion

The results of this study show that Japanese flounder larvae are able to feed on inert diets from first feeding. Total substitution of live foods by artificial diets may be possible if these diets are made digestible to these young larvae. This could be possible by designing microencapsulated diets with lipid walls that are digested by the larvae, and by the addition of exogenous enzymes in the microencapsulated feeds as described in a previous report [11]. Particle size also proved to be of paramount importance for feed ingestion by Japanese flounder larvae.

6. Acknowledgements

This research paper is an extract from a previously unpublished chapter from the author´s PhD thesis carried out at Kagoshima University, Japan. The publication of this work was supported by Instituto Nacional de Pesca, Guayaquil, Ecuador, and Proyecto Prometeo, Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (Senescyt), Republic of Ecuador.

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