

# Advances in Marine Fish Larvae Diets

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## Abstract

During the past three decades, enormous efforts have been made to develop microdiets (MD) to replace live feed, both rotifers and *Artemia*, as complete or partial replacements for marine fish larvae. However, although, there were substantial achievements in reducing the reliance on live feeds and weaning the larvae earlier onto MD, still, in most species, MD cannot completely replace live feeds.

There are several reasons why MD cannot, at this stage, completely replace live feed. Nutritional profile for marine fish larvae is yet to be completely defined. Although lipids and fatty acid requirements are known (to a degree), very little work was carried out to define the protein / amino acids, mineral and vitamins.

Chemical and physical properties are playing a crucial role with the behaviour of MD particle in the water, attractability, leaching, ingestion and ingestion in the larvae gut. However, very little attention was giving to these issues.

In recent years new diet manufacturing methods were adopted with potentially better particle properties. These methods will be discussed.

The best dry MD is as good as the method it is dispensed into the larvae tank. Compared to feeding systems and methods for on-growing fish, larvae feeding systems were not given much attention from both the scientific and commercial sectors. Only a handful of automated MD feeding systems exists and almost no scientific papers were published. The presentation will review the current available systems and requirements from MD feeding system.

## Introduction

During the past three decades, enormous efforts have been made to develop microdiets (MD) to replace live feed, both rotifers and *Artemia*, as complete or partial replacements for marine fish larvae (Kolkovski, 2004; Koven *et al.*, 2001). However, although, there were substantial achievements in reducing the reliance on live feeds and weaning the larvae earlier onto microdiets, still, in most species, microdiets cannot completely replace live feeds.

Although weaning the larvae from *Artemia* onto a MD can be achieved at metamorphosis in many species (Curnow *et al.* 2006a,b; Koven *et al.*, 2001; Foscarini, 1988; Hardy; 1989), the early introduction of prepared diets as the sole replacement for live food has met with limited success (Fernández-Díaz and Yúfera, 1997; Rosenlund *et al.*, 1997; Adron *et al.*, 1974; Barnabe, 1976; Kanazawa *et al.* , 1989; Appelbaum and Van Damme, 1988; Walford *et al.*, 1991). A clear example of the superiority of live food over commercial microdiets was demonstrated by Curnow *et al.* (2006a,b). Barramundi (*Lates calcarifer*) larvae development was affected by rearing protocols, with co-feeding rotifers and commercial diet allowing complete replacement of *Artemia*. However, by including *Artemia* in the protocol with one of the commercial MD, survival was significantly improved. Furthermore, feeding protocols with earlier weaning from rotifers resulted in significantly reduced growth and survival. (Curnow *et al.*, 2006a)

The efficiency of utilisation of feed particles (either live or inert) by marine larvae is affected by many external and internal factors. Primarily, the searching, identification and ingestion processes are influenced by physical and chemical factors including colour, shape, size, movement and olfactory stimuli at a molecular level.

Substances secreted by live food organisms that act to stimulate a feeding response belong to a group of chemicals known as ‘feed attractants’ and some have been specifically identified for larvae (Kolkovski *et al.* , 1997). Moreover, these physical and chemical factors affect the palette and influence the ingestion process, which is the precursor to the digestion process. Digestion involves secretion of enzymes, peristaltic movements and after larvae metamorphosis, acid and bile salt secretions. The assimilation and absorption process begins after the food particle is digested and broken down into more simple molecules that can pass across the gut lining. This is further facilitated by the development of brush border and microvilli as well as protein transporters and other transport mechanisms. Moreover these chemical and physical factors affect

the soft palette and influence the ingestion process, which is the precursor to the digestion process.

### **Feed Identification and Ingestion**

The feeding process (Fig. 1, modified from Mackie and Mitchell, 1985):

There are several steps in the larval process of finding and ingesting food particles:

- 1.) General and not specific reaction, initiation of search movements involving chemical and electrical stimuli.
- 2.) Identification of the food particle location involving chemical stimuli.
- 3.) Close identification of the food particle, involving chemical and visual stimuli.
- 4.) Tasting and/or actual feeding requiring chemical stimuli (taste buds).

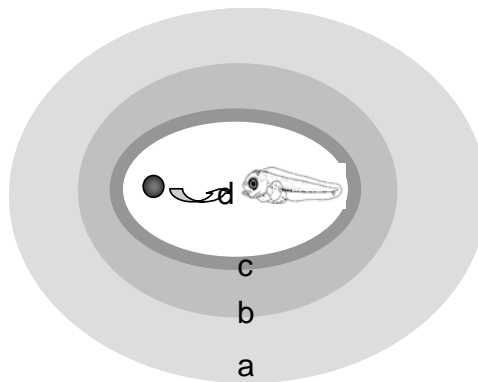


Fig. 1. The feeding process (modified from Mackie and Mitchell, 1985).

- a. General and not specific reaction, initiation of search movement - chemical and electrical stimuli; b. Identification of the food particle location - chemical stimuli; c. Close identification of the food particle - chemical and visual stimuli; d. Tasting and/or actual feeding - chemical stimuli (taste buds).

Various substances, such as free amino acids, nucleotides, nucleosides and ammonium bases, are released from organisms that are prey for fish larvae and are potent inducers of feeding behavior in marine (Knutsen, 1992; Doving and Knutsen, 1993, Kolkovski *et al.* 1997) and freshwater fish larvae. Synergistic relationship was reported between several amino acids (glycine, alanine, arginine) and the ammonium salt betaine, which when combined produced a stronger effect than

the sum of the individuals. Several amino acids as well as other substances were also found to be active as feed attractants (Table 1).

Tabla1. Amino acids as feed attractant in marine organisms (from Kolkovski, 2001)

|  |   |   |
|--|---|---|
| Rainbow trout <i>Salmo gairdneri</i>                 | Mixture of L-amino acids  | Adron and Mackie, 1978  |
| Atlantic salmon <i>Salmo salar</i>                   | Glycine   | Hughues, 1990   |
| Sea bass <i>Dicentrarchus labrax</i>                 | Mixture of L-amino acids  | Mackie and Mitchell, 1982   |
| Pig fish <i>Orthopristis chrysopterus</i>            | Glycine, Betaine  | Carr et al. 1977, 1978  |
| Red sea bream <i>Chrysophrys major</i>               | Glycine, Betaine<br>Glcine, Alanine, Lisien,<br>Valine, Glutamic acid and Arginine  | Goh and Tamura, 1980<br>Fuje <i>et al.</i> , 1981<br>Ina and Matsui, 1980 |
| Gilthead sea bream <i>Sparus aurata</i>              | Glycine, Betaine, Alanine, Arginine   | Kolkovski <i>et al.</i> , 1997  |
| Turbot <i>Scophthalmus maximus</i>                   | Inosine and IMP   | Mackie and Adron, 1978  |
| Dover sole <i>Solea solea</i>                        | Glycine, Betaine<br>Glycine, Inosine, Betaine                                       | Mackie <i>et al.</i> , 1980<br>Metaillet <i>et al.</i> , 1983             |
| Puffer <i>Fugu pardalis</i>                          | Glycine, Betaine  | Ohsugi <i>et al.</i> , 1978   |
| Japanese eel <i>Anguilla japonica</i>                | Glycine, Arginine, Alanine, Proline   | Yoshii <i>et al.</i> , 1979   |
| Cod <i>Gadus morhua</i>                              | Arginine  | Doping <i>et al.</i> , 1994   |
| Herring <i>Clupea herangus</i>                       | Glycine, Proline  | Damsey, 1984  |
| Glass eel <i>Anguilla anguilla</i>                   | Glycine, Arginine, Alanine, Proline<br>Alanine, Glycine, Histidina, Proline         | Mackie and Michell, 1983<br>Kamstra and Heinsbroek, 1991                  |
| Lobsters <i>Homarus Americanus</i>                   | Glutamate, Betaine, Taurine,<br>Ammonium chloride                                   | Corotto <i>et al.</i> , 1992  |
| Western Atlantic ghos crac <i>Ocyropode quadrata</i> | Butanoic acid, Carboxylic acid,<br>Trehalos, Carbohydrates, Homarine,<br>Asparagine | Trott and Robertson, 1984   |
| Freshwater prawn <i>Marobrachium rosenbergii</i>     | Taurine, Glycine, Trimethylamine,<br>Betaine  | Harpaz <i>et al.</i> , 1987   |
| Abalone <i>Haliotis discus</i>                       | Mixture of L- amino acid and<br>lecithin  | Haradae <i>et al.</i> , 1987  |

A practical way to increase the ingestion rates of microdiets would be to incorporate these substances as extracts or hydrolysates into the diet. Kolkovski *et al.*, (2001) tested the effect of krill hydrolysate as a feed attractant on yellow perch *Perca flavescens* and lake whitefish *Coregonus clupeaformis*, by coating commercial starter diet with 5% krill hydrolysate. Fish fed

the coated diet experienced similar growth to fish fed live *Artemia* and significantly higher (31%) growth than fish fed the control diet (Fig. 2).

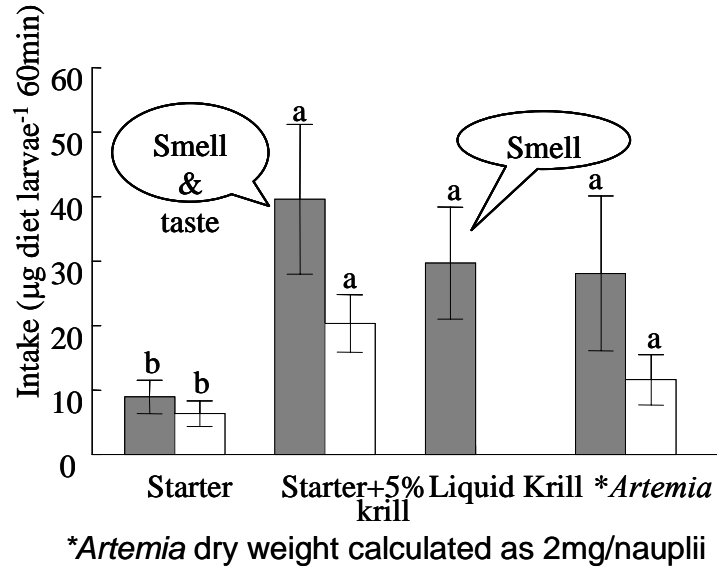


Fig. 2. Effect of krill hydrolysate on ingestion rates of yellow perch and whitefish (Kolkovski et al., 2000).

Gray bars - Yellow perch (Average wet weight- 42±4 mg), White bars – whitefish (Average wet weight- 13.5±2 mg).

Furthermore, a recent experiment was conducted to determine whether the method of hydrolysate incorporation in microdiets affected growth of yellowtail kingfish (*Seriola lalandi*) larvae. Krill hydrolysate was compared coated or incorporated into the diet (Kolkovski *et al.*, 2006). Growth rates of larvae fed coated-diet were significantly higher than larvae fed krill hydrolysate incorporated diet. Both diets (incorporated and coated) performed significantly better than the control diet with no hydrolysate (Fig. 3). Other hydrolystaeas such as squid hydrolysate were also found to be effective in increasing both ingestion and growth (Kolkovski *et al.*, 1997, 2000; Lian *et al.*, 2008, Lian and Lee, 2003). Currently, several commercial microdiets includes different hydrolysates such as krill, fish and squid hydrolysates in their formulation.

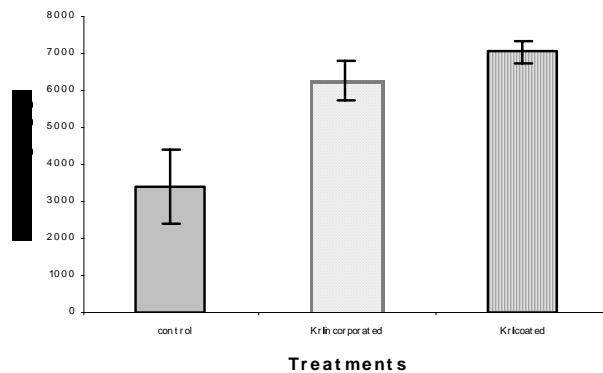


Fig. 3. Effect of krill hydrolysate inclusion method on growth of yellowtail kingfish *Seriola lalandi*. Incorporated krill – krill hydrolysate was mix at 3% (DW) with other ingredients, Krill coated – krill hydrolysate was coated the MD particles.

### ***Amino acids vs. hydrolysates as feed attractants: pros and cons.***

#### *Amino acids*

- Only the L-isomers have been found to be active as feed attractants
- Various combinations of amino acids have been found to have a positive effect on various fish species
- Synergistic effects were associated with many combinations of amino acids and other substances such as ammonium salts
- Increasing the concentration of amino acids (when added to the water) was found to have positive effects on feeding, range from  $10^{-8}$  M to  $10^{-2}$  M.

#### *Hydrolysates*

- Hydrolysates act as feed attractants as they contain digested protein components such as free amino acids and peptides.
- Concentrations of extracts and/or hydrolysates made from aquatic animals are harder to quantify than amino acids. However, concentrations that are found to have a positive effect on feeding range from  $10^{-2}$  to  $10^{-10}$  g/l (when added to the water).
- In most cases, when incorporated into the diet, the concentration of hydrolysates and extracts released into the water was not determined.
- As a ‘general rule’, protein fraction weight between 1000 and 10,000 Dalton is found to have a positive effect on feeding. Hydrolysates have also, positive aspect of being protein source

especially for non-stomach larvae. However, the ratio of hydrolysate / whole protein should be carefully look at due to the negative effect of inclusion of high percentage of hydrolysates to microdiets (Kolkovski, 2001)

#### *Presentation of feed attractants to fish larvae*

- The addition of attractants directly into the water uses large amount of these substances, but maintains a constant concentration.
- Coating the diet particle results in unknown leaching rates, but can contribute to higher palatability and more specifically identifies particles as food.
- Incorporation into the diet, as part of the protein source also results in an unknown leaching rate (depending on the microdiet type), however only a low amount of attractants are needed, part of the protein source in the diet is replaced, and digestion and assimilation is improved.

### **Digestion**

The basic capacity and rates of hydrolysis and transport of specific nutrients within the fish larvae intestine is qualitatively and quantitatively set in genetic ‘memory’ to correspond to a natural diet. At first feeding, the digestive tract in most fish species contains the enzymes related to digestion, absorption and assimilation of molecules such as proteins, lipids and glycogen (Kolkovski, 2001). However, larval enzyme activity has been found to be relatively low when compared to adult fish (Cousin *et al.*, 1987). Each enzyme develops independently during ontogenesis, with variation related to fish species and temperature and the suitability of food type (Cahu and Zambonino Infante, 2001).

It has been proposed that exogenous enzymes from live prey could directly aid in larval digestion or activate the zymogens present in larval gut, thus increasing digestion and growth rates (Dabrowski, 1979; Lauf and Hoffer, 1984). The mechanisms through which exogenous enzymes could aid or stimulate the digestive process are not clearly understood. Live food organisms also contain gut neuro-peptides and nutritional ‘growth’ factors that may enhance digestion (Kolkovski *et al.* 1997, Kolkovski 2001, Ronnestad *et al.*, 2007). These substances are frequently omitted in formulated diets. However, the level of contribution of live food organisms to the digestion process is debatable.

MD contain proteins and other ingredients that are relatively difficult for larvae to digest. Based on the hypothesis that larvae are lacking the digestive capacity to breakdown dry particles and complex proteins, the inclusion of different digestive enzymes, especially proteases, in the microdiets has been shown to be beneficial for some species (such as sea bass and sea bream) while its benefits have not been conclusively demonstrated for other species (Kolkovski, 2001).

Therefore, rather than adding digestive enzymes to the MD, the inclusion of pre-hydrolyzed proteins (hydrolysates) in the MD was tested. Amino acids may increase the secretion of certain hormones, such as somatostatin and bombasin, which also stimulate the secretion of pancreatic enzymes (Chey, 1993; Kolkovski *et al.*, 1997). Live feeds contain large amounts of free amino acids, which may stimulate the secretion of trypsin (Dortch 1987; Hamre *et al.*, 2002). Cahu and Zambonino-Infante (1995) reported increased trypsin secretion in sea bass larvae fed a mixture of free amino acids in their MD.

However, the supplementation of hydrolyzed protein and/or free amino acids or short peptide gave mixed results depending on the percentage of inclusion, fish species and larval age (Table 2). Possible explanations for these results were suggested:

- Fast flow of short peptides and FAA through the gut, a flow that the larvae cannot handle in terms of FAA absorption (Kolkovski, 2001).
- Amino acid absorption rates and specific nutrient receptors progressively change as they pass through the gastrointestinal tract of the fish. Therefore, premature absorption of certain essential amino acids presented in the free form can obstruct the absorption of other essential amino acids, polypeptides or intact proteins (Hardy, 1991).

From these results it can be concluded that free amino acids and hydrolysates can only partially replace the intact proteins in fish larvae MD designed. As a general recommendation, the level of the hydrolysate should not exceed 30% of the total protein levels.



Table 2. Marine organisms hydrolysates used as feed attractant and/or protein replacement in microdets (updated from Kolkovski, 2001).

| Organism  | Tested on  | Reference                                       |
|---|--|---|
| <i>Balanus nauplii</i>                          | Herring <i>Clupea harengus</i>   | Dempsey, 1978                                   |
| Tubifex blood worm                              | Tilapia  | Iwai, 1980                                      |
| Short necked clam <i>Tapes japonica</i>         | Japanees eel <i>Anguilla japonica</i>  | Hashimoto <i>et al.</i> , 1968                  |
| Cod <i>Gadus morhua</i>                         | Hermit crab <i>Petrochirus diogenes</i>  | Hazlett, 1971                                   |
| Cod <i>Gadus morhua</i>                         | Glass ell <i>Anguilla anguilla</i>   | Kamstra and Heinsbroek, 1991                    |
| Abalone   | Spiny lobster <i>Panulirus interruptus</i>   | Zimmer-Faust <i>et al.</i> , 1984               |
| Dungeness crab <i>Cancer magister</i>           | Little neck clam <i>Protethace staminea</i>  | Pearson <i>et al.</i> , 1979                    |
| Pink shrimp <i>Penaeus duorarum</i>             | Spiny lobster <i>Panulirus argus</i>   | Reeder and Ache, 1980                           |
| Marine polychaete <i>Perinereis brevicirrus</i> | Red sea Bream <i>Chrysophrys major</i>   | Fuke <i>et al.</i> , 1981                       |
| Shrimps   | Rainbow trout <i>Oncorhynchus mykiss</i> and Atlantic salmon <i>Salmo salar</i>  | Mearns <i>et al.</i> , 1987                     |
| Krill <i>Euphausia pacifica</i>                 | Yellow perch <i>Perca flavescens</i> , Walleye <i>Stizostedion vitreum</i> , Lake whitfish <i>Coregonus clupeaformis</i> , | Kolkovski <i>et al.</i> , 2000, Kolkovski, 2001 |
| Krill <i>Euphausia pacifica</i>                 | Barramndi, <i>Lates calcarifer</i>   | Curnow <i>et al.</i> , 2006                     |
| Krill <i>Euphausia pacifica</i>                 | American lobster <i>Homarus americanus</i>   | Floreto <i>et al.</i> , 2001                    |
| Krill <i>Euphausia pacifica</i>                 | Black tiger shrimp <i>P. Monodon</i>   | Smith <i>et al.</i> , 2005                      |
| Mussel <i>Mytilus edulis</i>                    | Gilthead sea beam <i>Sparus aurata</i>   | Tandler <i>et al.</i> , 1982                    |
| Fish (non specific)                             | Black tiger shrimp <i>P. Monodon</i>   | Smith <i>et al.</i> , 2005                      |
|   | Largemouth bass <i>Micropterus salmoides</i>   | De Oliveira and Cyrino, 2004                    |
| Squid   | Summer flounder, <i>Paralichthys dentatus</i>  | Lian <i>et al.</i> , 2003, 2008                 |

## Diet Manufacturing Methods

Several microdiet manufacturing methods are currently being used:

- (1) microbound diets (MBD) (Fig. 4),
- (2) micro-coated diets (MCD) and
- (3) micro-encapsulated diets (MED) (Fig. 5) and,
- (4) marumerisation (MEM) (Fig. 6)

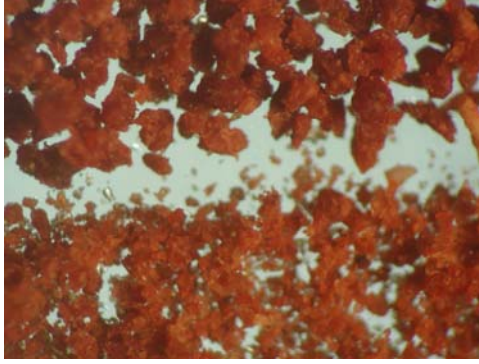


Fig. 4. Microdiets manufactured by micro binding (MBD) technique.

Fig. 5. Microdiets manufactured by micro encapsulation (MED) technique  
photo Manuel Yufera, CICS, Cádiz, Spain).

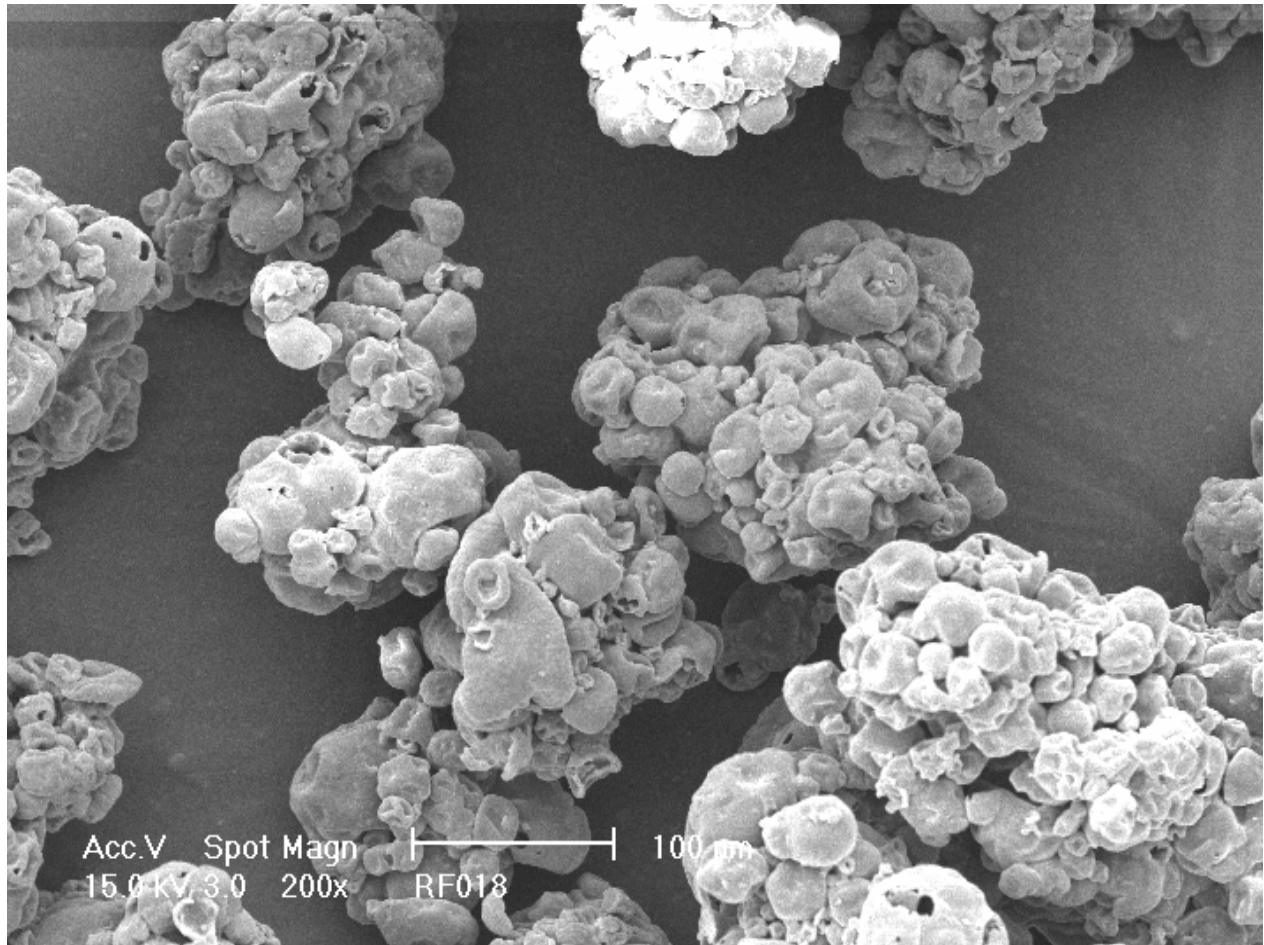


Fig. 6. Microdiets manufactured by merumeasaton (MEM) technique (photo Bernard Devresse, BernAqua Belgium)

### **MBD**

Currently, the manufacturing process of MBD's is the simplest and most commonly used method of preparation. It consists of dietary components held within a gelled matrix or binder. They do not have a capsule and it is suggested that this facilitates greater digestibility and increased attraction through greater nutrient leaching (Kolkovski, 2001, Yúfera *et al.*, 2003; Kolkovski, 2006). Some commercial microdiets are manufactured using extrusion and then crushed and sieved to the required particle sizes. All the ingredients are ground, mixed with a binder such as gelatine, alginate, zein, carrageenan and carboxymethyl-cellulose, activated by temperature or chemically (López-Alvarado *et al.*, 1994; Kolkovski, 2004, 2006b, Koven *et al.*, 2001) and then dried (drum drying or spray drying), ground and sieved to the required size.

## MCD

MC method is based on coating or binding small MBD particles to reduce leaching (López-Alvarado *et al.*, 1994; Baskerville-Bridges and Kling, 2000, Onal and Langdon, 2004). The coating layer is usually lipids or lipoproteins. This method is not often use in commercial processes.

## MED

MED particles are made by using several different techniques. The particle usually has a membrane or capsule wall, which separates dietary materials from the surrounding medium (Fig. 5,6). The capsule wall helps maintain the integrity of the food particle until it is consumed preventing leaching and degradation of the nutritional ingredients in the water. However, this attribute may restrict leaching of water-soluble dietary components and therefore reduce the larvae's attraction to the food particles (Yúfera *et al.*, 2003, Kvale *et al.*, 2006). The capsule wall is also thought to impair digestion of the food particle (Yúfera *et al.*, 1998, Kolkovski, 2006) (Fig 5). There are several methods for micro-encapsulation. These include chemical processes and mechanical processes. In chemical processes, the capsules are made within a liquid, usually stirred or agitated. The capsules are formed by 1) spraying droplets of coating material on a core ingredients, 2) capsulating liquid droplets containing the nutritional ingredients by spraying into gas phase, 3) creating gel capsules by spraying droplets, containing the nutritional ingredients and a binder, into liquid solution that activate the binder or by polymerization reaction at a solid/gas or 4) liquid interfaces. Protein cross-link (Yufera *et al.*, 1998) involves several stages of mixing and washing with organic solvents resulting in a very expensive diet process, as well as potentially toxic. These methods never resulted in good growth rates due to the larvae inability to digest and assimilate the particles as well as the high ratio of non-nutritional ingredients mainly the capsule, to the essential nutrients (Yufera *et al.*, 2005).

Another method, complex coacervation, involves mixing and activating, using electrical charges, two liquid phases differing in their viscosity resulting in very small capsules. These capsules then bind to create a larger capsule containing hundreds or thousands of microcapsules (Thies, 2007) (Fig. 6).

Mechanical encapsulation involves processes such as spray drying, fluidized bed drying, cold micro-extrusion marumerization (MEM) and particle-assisted rotational agglomeration (U.S. patent 5,851,574). The last two techniques have gained attention in the past few years with already commercially available diets produced with these methods. Initially developed for pharmaceutical processes, these methods involve purpose built machines. MEM is a two-step process of cold extrusion followed by marumerization (spheronization). The process has the capability of producing particles from 500 - 1,000  $\mu\text{m}$  and greater. Particle-assisted rotational agglomeration (PARA), which is a single step process capable of producing particles from 50 – 500  $\mu\text{m}$  that are lower density than particles produced by the MEM method due to the fact that the extrusion step is avoided. The method is based on a spinning disk (marumerizer). A wet mash of the ingredients is put into the marumerizer with or without inert beads. The rotation movement of the disk brakes down the mash into smaller spherical particles. The diameter of the particles depends on several factors including the disk rotation speed, the inert beads, the raw ingredients etc (Barrows and Lellis, 1996, 2006).

## **Microdiet characteristics**

### *Leaching*

As mentioned above, one of the problems of MBD particles and in fact most of the microdiet type particles is the high leaching rate of amino acids. Kvale *et al.*, (2006) reported leaching of protein molecules (9-18 kD) after 5 minutes immersion in water (3% NaCl, 12°C) at a rate of 80-98%, 43-54% and 4-6% for agglomerated, heat coagulated and protein encapsulated MD. Yufera *et al.*, (2003) determined the rate of different amino acids leaching from both MBD and MED. The authors found contrary patterns between the two diet types. While hydrophilic amino acids leached the most from MBD, hydrophobic amino acids were found to leach from MED particles at a higher rate (Fig. 7). The leaching rates of the two diets were also significantly different. For instance, 70% of free lysine leached from MBD particles after less than 5 minutes, while less than 7% leached from MED particles after 60 minutes (Fig. 8). López-Alvarado *et al.*, (1994) tested the leaching rates from several different MD made with different techniques and found similar results.

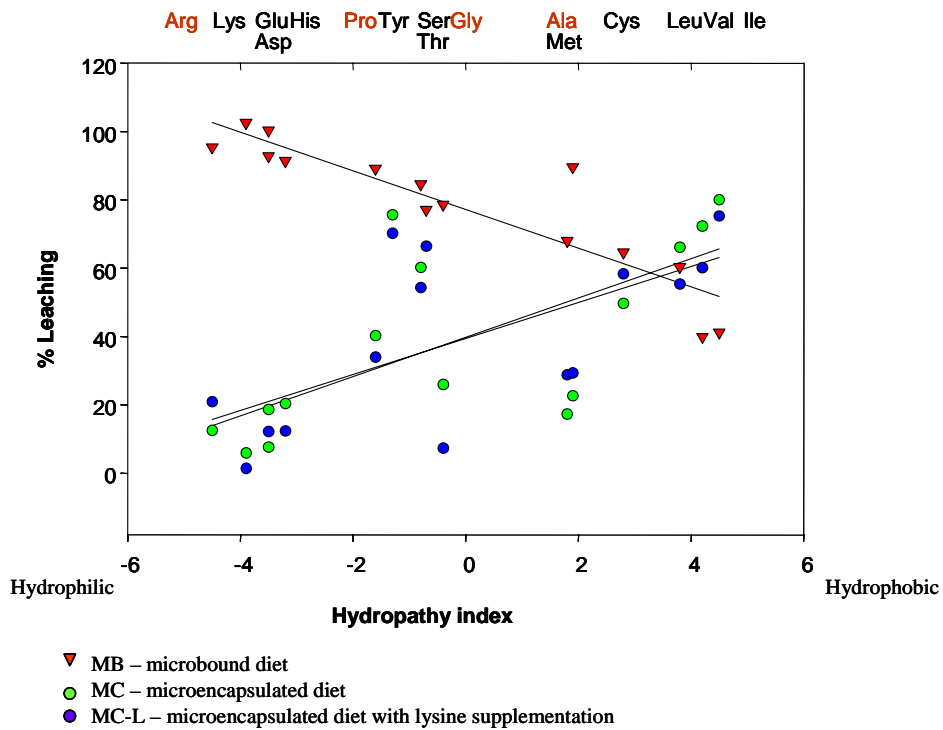


Fig. 7. Percentage of FAA leached after 60 min of immersion in water in relation to the hydropathy index (Yufera *et al.*, 2002)

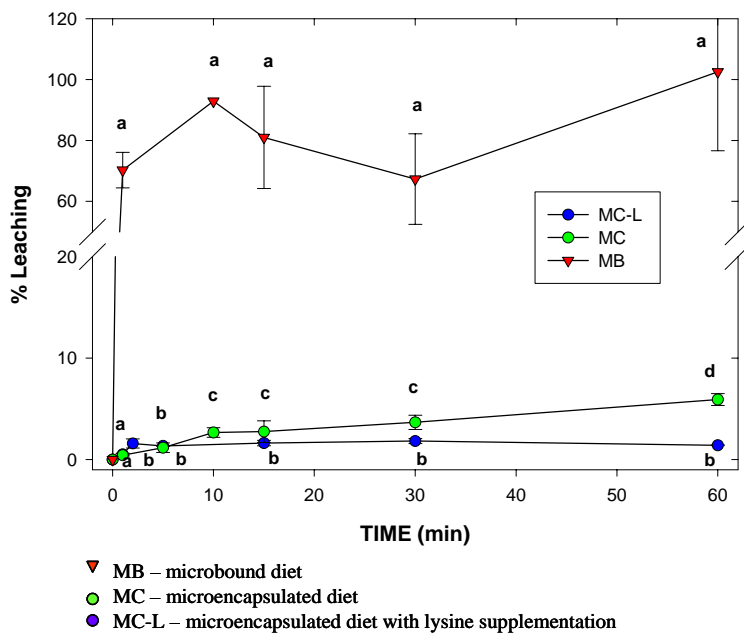


Fig. 8. Leaching pattern of Lysine during 60 min of immersion in water (Yufera *et al.*, 2002)

A diet particle needs to achieve a fine balance between leaching amino acids and other nutrients to act as feed attractant and digestibility of the particle to suit the undeveloped larvae digestive system. A particle that will be hard and leach resistant will also present a challenge to the larvae digestive system, whilst, a particle that will digest easily in the gut will also disintegrate relatively fast in the water (Kolkovski, 2006a, Yufera *et al.*, 2005)

### *Buoyancy*

One of the most significant problems concerned with microdiet particles is their negatively buoyant inert state. However, there are very few scientific papers investigating this issue. MBD particles don't move like live zooplankton such as *Artemia*. This specific movement act as a visual stimulus for increased feeding activity (Kolkovski *et al.*, 1997). Furthermore the particles sink to the bottom of the tank where they are no longer available to the larvae and accumulate there, leading to bacterial proliferation and deterioration of water quality. This further necessitates the need to effectively wean the larvae onto the MBD, in order to both modify their digestive capacity and their feeding behaviour. A change in behaviour is illustrated by the larvae's ability to recognize the inert particles as food and to more actively hunt for them during a relatively smaller window of opportunity, as the particles pass down through the water column. Figure 9 illustrate the sinking rates of several commercial microdiets (Jackson and Nimmo, 2005). Different attempts have been made to increase the time the microdiet particle spends in the water column including increasing buoyancy by adjusting and modifying oil levels, manufacturing methods and also using rearing systems with up welling currents (Kolkovski *et al.*, 2004, Teshima *et al.*, 2004). Knowledge of sinking and leaching rates of microdiets can and should be used to optimise feeding time in the larvae tank. The faster the diet particle sinks the shorter the feeding intervals should be coupled with smaller quantities of diet, in short, feeding less more often.

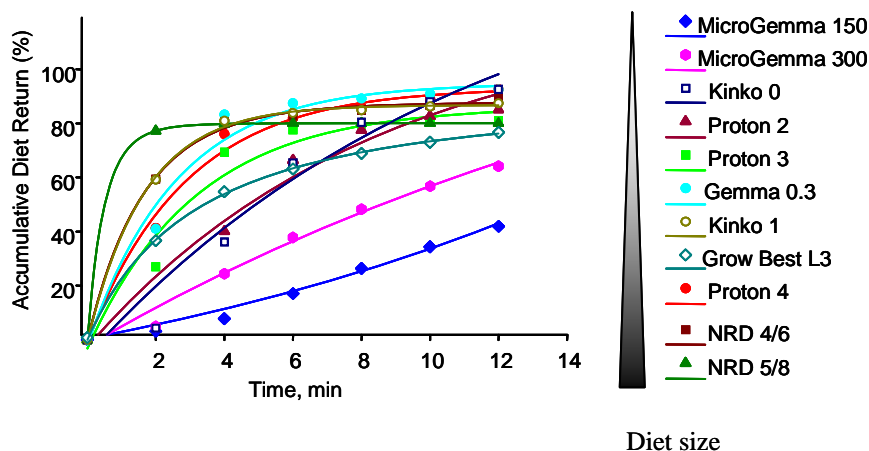


Fig. 9. Sinking patterns of commercial diets (Jackson and Nimmo, 2005)

### Weaning and co-feeding methods

An important factor influencing the larvae's acceptance of a microdiet, which affects both their growth and survival, is the weaning process. In the past, early weaning has led to poor growth and inferior quality larvae with an increased risk of skeletal deformities (Cahu and Zambonino Infante, 2001). Recent advances in microdiet formulation have considerably reduced the pre-weaning period allowing the introduction of specific larval diets to marine finfish culture as early as mouth opening (Cahu and Zambonino Infante, 2001). 'Co-feeding' weaning protocols, simultaneously using inert and live diets, allow an earlier and more efficient change over period onto microdiet from live feeds (Hart and Purser, 1996; Daniels and Hodson, 1999; Koven *et al.*, 2001, Curnow *et al.*, 2006, a,b). This method provides higher growth and survival than feeding solely live feeds or microdiets (Kolkovski *et al.*, 1995). Early co-feeding of an appropriate microdiet will improve larval nutrition and can condition the larvae to accept microdiet more readily, thus preventing an adverse effect on subsequent growth following weaning (Rosenlund *et al.*, 1997; Canavate and Fernandez-Diaz, 1999; Cahu and Zambonino Infante, 2001; Kolkovski, 2001). Curnow *et al.*, (2006 b) demonstrated the effect of different weaning and co-feeding treatments on growth and survival of barramundi *Lates calcarifer* larvae (Fig. 10). The authors found that early weaning before the larvae had adequately developed, as well as diet type and quality not only influenced growth and survival, but also the occurrence of cannibalism. They concluded that Co-feeding barramundi larvae on microdiet should be started no earlier than 3



days prior to stomach differentiation and be continued post metamorphosis. This method improves growth by 25–30% over the previous standard method, and mortality during weaning was reduced from 5% to 1% (Bosmans *et al.*, 2005).

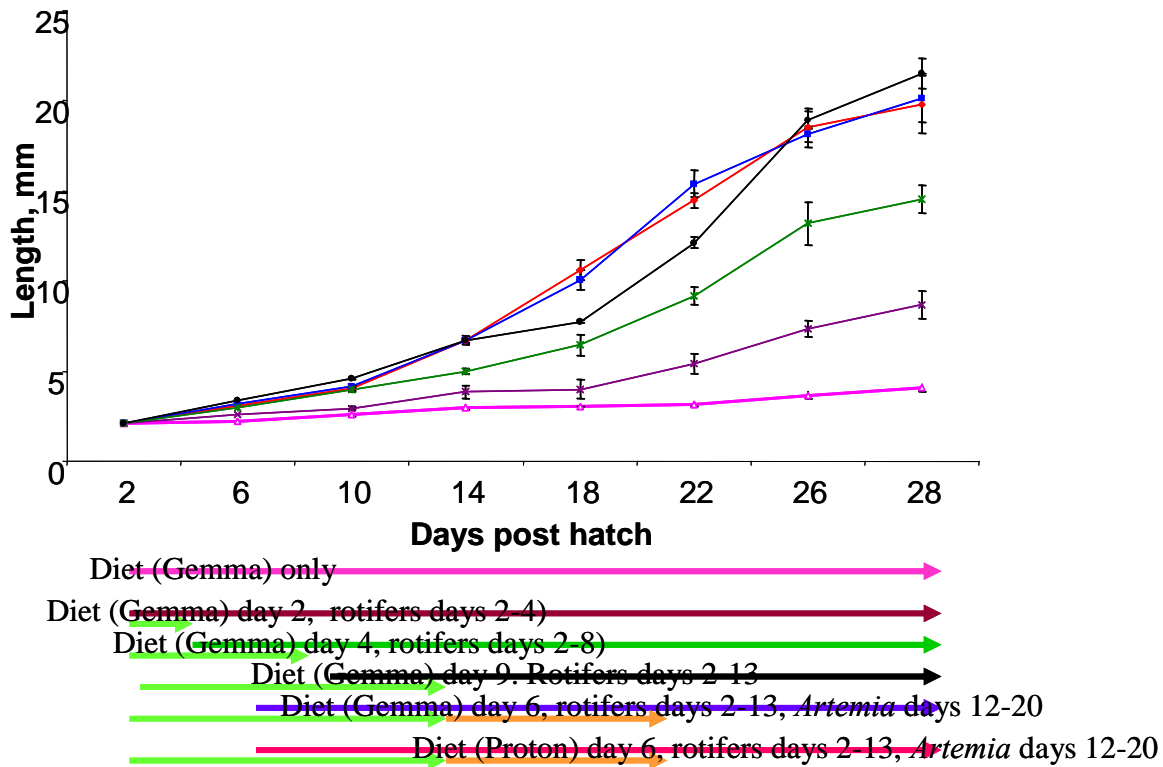


Fig. 10. Effect of various feeding protocols on Barramundi *Lates calcarifer* larvae length (Curnow *et al.*, 2006a)11. Automatic Microdiet Dispenser (AMD, Department of Fisheries, Western Australia), a – side view, b – bottom view, static plate secure with bolts while the moving plate above it been hold by the solenoid piston.

## Feeding system

The digestibility and nutritional qualities of the commercially available MD are now becoming better and better due to continuous R&D, as motioned above. However, none of these commercial MD's are used solely without *Artemia* (not to mention without rotifers). Part of the reason is the MD distribution or the manner served to the larvae. The best dry MD is as good as the method it is dispensed into the larvae tank.

Compared to feeding systems and methods for on-growing fish, larvae feeding systems were not given much attention from both the scientific and commercial sectors. Only a handful of

automated MD feeding systems exists and almost no scientific papers were published (Papandroulakis *et al.*, 2002).

Hand feeding is the simplest and still most widely used. Hand feeding is usually made using small devices (spoons, salt-boxes) with relatively long periods between feeding events (30 to 60 minutes). Covering long photoperiods is difficult due to labor and logistics involving long feeding periods sometimes over 24 hrs. Due to the high larval metabolic rates and the demand for continuous feeding, the result is insufficient benefits from a relatively expensive product.

It has been recognized that European hatcheries (and in fact, any modern intensive hatchery) had a strong need for automation in all the production processes. Not only would it generate labor savings, but also it will secure the production protocols and bring more repeatability to every step of the processes (Leclercq, D., 2004). In this respect, intents were made to develop automatic dispensers of microparticles that would be precise in their distribution and easy to use in hatcheries.

### *Dosage system*

The first requirement from a mechanical MD dispenser concerns its capacity to deliver one stable quantity per feeding event.

Well-known belt feeders (FIAP Aquaculture, Denmark), driven by a motor or by a clock, are not capable to split a daily ration in equal aliquots of feed. They are handy and cheap but not actually built for MD particles resulting in the microparticle sticking to the belt, especially at humid conditions.

A cleverly designed system was developed in Australia ('AMD' [automatic Microdiet Dispenser], Department of Fisheries, Western Australia, Fig 11a,b) which its dosage system is based on the opening of a sluice-valve quickly moved by the mean of a simple solenoid allowing for a constant quantity of feed to be delivered at each feeding event. Cleaning the feeder is a very simple and quick process. Air from the hatchery main is used with a built-in spreader.

### *Delivery to the rearing tank*

Once a reliable dose is established, its repetition over the tank surface or in the tank volume is necessary to increase the larvae particle interaction (i.e. before it sinks to the bottom of the tanks and become unavailable to the larvae).

The dose delivery can take place directly above the water surface. However, there is a risk of aggregation of particles to small packs sticking together and immediately sinking to the tank bottom.

To avoid this, Raunes, a cod hatchery in Norway, has developed an intermediate vessel where the MD is mixed with water and further distributed into the water column at different points of the tank. These are commonly referred to as “spiders”. The vessel volume is only a few liters and the applied flow (part of the tank water intake) allows for 2-3 minutes of residence time in the vessel. In this vessel, the dose is being delivered into the flow of water by any dispenser and the microparticles are being separated and dispersed by the strong water movements into the vessel. The suspended particles are then pushed into the tanks through the “spider legs”, these being made from a number (6-12) of small rigid plastic (2-3mm internal diameter) pipes. The lower end of each of them is being set into the water column (2-3cm below the tank surface). This way of dispersion of the microparticles is very efficient especially in large tanks (4-10m<sup>3</sup> or more). It avoids trapping the microparticles in the surface skimmers, which are often in use to capture the oil-film at the surface of the tanks. This way of dispersing the MD particles is most efficient, however, due to the strong water movement it may also cause very strong leaching of the nutrients. The extent of this problem is yet to be determined.

Another way of spreading the MD over the surface is included in the AMD. An air-blade is formed under the dosage point (where the MD dose is delivered by the dispenser) and blows the light particles over an area that can reach 30-90cm. Once separated from each other by the air current, the particles do not intend to clump and conglomerate over the surface. This is simpler than the “spider” device described above, but requires tanks without skimmers, which would trap the microparticles before they sink.

### ***Fractioning of the daily ration into multiple events***

As mentioned above, fish larvae have a limited ‘window of opportunity’ to catch the MD particles before they sink to the bottom of the tank and become unavailable. It is also a current

practice in modern hatcheries to establish long photoperiod during the larval production (from 16 to 24 hours light). Therefore it is extremely important that the distribution of the MD be split over time in very frequent feeding events. This will give the larvae a chance to catch fresh microparticles in the water column.

In that respect the control panels of the dispensing systems should include the ability to have frequent feeding events. This is an essential role in establishing the distribution regime and potential success of the MD feeding strategy.

Some control panels allow the programming of the duration of one single event and the duration in between events (i.e. successive time laps with yes/no control on the feeder). This process allows for quantitative dosage through time but it requires permanent adjustment of the event duration, day after day, to cope with increasing quantities to be distributed to a tank as the biomass grows.

To allow fine distribution quantities of expensive feed over the photo period of the daily production cycle requires each feeding event to distribute very small quantities of feed. For the finest MD (50-200 $\mu$ ), quantities of as low as 100-300 mg of feed/event should be achieved. For a given feed ration, the permanent distribution at time laps of, as low as, one or two minutes will give far better results in both sparing the feed and tank cleanliness than sequences of larger distributions every 10 minutes or so (Leclercq and Kolkovski, personal observations). A continuous process would work as well (like what the belt feeders deliver with larger diets), but no equipment can, currently, deliver both continuous and precise dosage of the feed over time.

Therefore, using a control panel allowing for precise amounts related to a single feeding event with time laps between events being adjustable is a more advisable practice.

### ***Surroundings of the feeding device in the hatchery:***

Hatcheries are very wet environments with high humidity. This, coupled with the hygroscopic nature of MD, often, led to MD's failure to perform when automated feeders were used. It should be strongly advised to use tightly closed containers at any stage to store the MD once the bag has been open. Silica gel bags, often replaced and dried again, can be used efficiently in the containers if there is a high risk of humidity in the storage place. The feeder's hoppers should

have splash resistance and the lid should be closed as soon as the hoper is filled with a new quantity of feed.

Water spray from air diffusers in the tank, is also generating moisture and MD starts, inevitably, to stick to the feeder and eventually leads to microbial development. Feed dispensers should be placed at a sufficient height over the water surface (>25cm, if possible) and away from any aeration/oxygenation device, which generates clouds of tiny water bubbles.

Cleaning of the device should be easy and quick. Dismounting should be limited but sufficient to clean all parts susceptible to retain MD particles over time. When dismounting is necessary, it should be a simple exercise for one operator alone (some feeders requires 4 hands just to clean them). High protein percentage used in the MD leads to extremely quick biodegradation when the MD accumulates moisture (normal moisture content in MD is 7-12%). This means that feed dispensers should be looked at very closely from a hygienic point of view. If its built-in qualities make it easy to dry clean, a quick air-jet with pressure air every day (if available) may sweep the dust off the dispenser and keep it clean. If pressurized air isn't available on site or if the dispenser keeps moist diets sticking on its parts, a complete wash and successive drying will be necessary every day. It must be possible to disconnect the dispenser from where it works for maintenance and cleaning (if not, it will become dirty and loaded with bacteria and fungi). Keeping the dispenser clear of any accumulation of diet is also necessary to keep it working to a reliable standard. Stacked MD particles may impair the proper rotation of the moving parts of the dispenser reducing the amount being fed after time.

There is a possibility of a feeder falling into a tank. Therefore, the feeder support should be accessed easily to reduce this risk. The supplied current to the feeder should be low (12-24v) to prevent any electrocution hazards. Furthermore, if the feeder falls into a tank, some commercial product will continue to work having some water resistance. Other will immediately shortcut and be impossible to restart.

## **Future directions**

The lack of suitable microdiet that can replace live feed and be manipulated to address the nutritional requirements of fish larvae is still the bottle neck in marine fish larvae nutritional studies. An integrative approach needs to be taken in the development of microdiets for fish

larvae taking into account the physiology and ontogeny of the larval digestive system, and nutritional requirements (lipids, proteins, vitamins and trace elements) as well as technology (leaching, sinking, binders, feeding and rearing systems). Microdiet manufacturers need to focus on better ingestion, digestion and assimilation of a balanced nutrient profile that is provided using an all-encompassing approach. To date, larval nutritional requirements are only partially identified and much is still unknown.

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