9. Fish feed and feeding techniques

First feeding

At hatching, fish larvae are not yet completely formed and, among other things, lack functional eyes and mouth. Moreover they do not have an active swimming behaviour. In the first three to six days after hatching, and depending on water temperature, the fish larva therefore relies only on its yolk sac reserves as food source. At the end of this period the young fish has developed functional eyes, which are recognisable by their dark colour, its mouth has opened and the digestive tract, though still primitive, can now assimilate food. Then, its swimming behaviour becomes active and the animal is thus able to keep a horizontal position. At this stage the post-larval stage begins and the young fish starts feeding on live preys, such as rotifers or brine shrimp nauplii (the latter only a first feed in the case of seabass). These live feeds are supplied on time to the larval tank (see annex 17 and 18 for feeding regime of seabass and gilthead seabream).

The onset of the first larval feeding is a crucial step in the young fish life: if something goes wrong, starvation quickly kills a weakened animal. Starvation is actually a major cause of larval mortality. Therefore it is important to help them to overcome this delicate and key phase. The main actions to be taken are the following:

- Maintain the larval population uniformly dispersed in the water column with the help of a gentle aeration, avoiding any mechanical o physical stress until the larvae reach the proper combination of size, organ development and behaviour.
- Water temperature, a crucial factor for a correct organ development, should be kept within ±0.2°C from the chosen value and close to the spawning/hatching temperature during the first week and after that the temperature increase should be kept within some fractions of a degree per day until the final rearing temperature is reached.
- At least during the first ten days after feeding has started a high prey density increases the chance for the fish to approach and gulp some rotifers or artemia nauplii, improving significantly their survival possibilities. Prey density at this stage should be kept at 5 to 10 rotifers/ml and regularly checked throughout the period when the lights are on to adjust subsequent distribution accordingly.
- Take into consideration the differences between seabass and gilthead seabream larvae in feeding behaviour, food ingestion and food assimilation: large fluctuations of prey density are to be expected in seabass populations due to their faster and greater ingestion rate than in the case of gilthead seabream, which are less active predators. See Annexes 17 and 18 for feeding protocols.
- Prey size at first feeding is of crucial importance for gilthead seabream. Because of their very small mouth, during the very first days they can only ingest preys smaller than 100 µm. Thus a ration composed almost exclusively of rotifers of a small-size-strain (50-100 µm juvenile and adult respectively) or of the juvenile rotifers of a medium-size-strain (70-130 µm) is essential. Later on, bigger rotifers (150-250 µm) can also be fed.
- The proper management of first feeding requires a close monitoring of the larval population: daily samples from each tank have to be collected and examined under the microscope to assess repletion rates (percentage of larvae having preys in their stomach) and number of prey per fish (see below for a detailed description of the sampling procedure). At the end of the first week of feeding, in a healthy population almost 100% of sampled fish should show some preys in their digestive tract, their quantity being directly linked to the mouth size. Table 12 gives the indicative figures of repletion rates and the number of ingested preys for both species. Annex 19 gives an example of a larval quality control form.
- Record also all feeding data on dedicated tables, (see Annex 20 for an example of tank file, Annex 21 for larval rearing unit daily feed distribution schedule, and Annex 22 for daily quantities of live food to be distributed during the week).
- To check all sector operations use a larval rearing sector daily workplan sheet as indicated in Annex 23.

Table 3.10 - Repletion rate and number of ingested preys per larva at first feeding of gilthead seabream and seabass larvae.

Age	Repletition rate	Actual ingested rotifer/ARTEMIA	
	%	Gilthead seabream	Seabass
3rd day	20-40	2-3	2-4
4th-5th day	70-90	4-8	7-12
6th-8th day	100	10-15	15-20
9th day	100	up to 50	up to 100
onwards			

Transition from live feed to artificial food

Feeding on live prey usually lasts 40 to 50 days, according to water temperature, species, rearing protocol and the opinion of the larval unit manager. The sooner the fish move to an artificial diet, the better in terms of savings on labour, overall costs and time. With this aim in mind, the timing of first dry food supply has been continuously anticipated in recent years, thanks also to new more elaborated artificial diets, including vitamins and immunostimulants, which fit better the larval requirements in terms of composition, size, buoyancy, and flavour. Special processing techniques can now blend high quality ingredients into micro-particles showing excellent stability in water, a slow sinking rate and attracting fish larvae better.

Before these advanced diets were available, fish were weaned on freshly prepared wet diets, formulated according to the nutritional requirements of other fish species. Special attention had to be given to the quality of the raw materials and integrators, as well as to their processing. A detailed description of such a wet diet (moist feed) and an example of feeding rates are presented in Annex 24.

The main difficulty encountered when trying to feed an artificial diet for the first time is how to stimulate fish to accept it, which is a time-consuming task since they are used to live preys. This delicate transition is indeed one of the main sources of larval mortality. In practice, at the beginning inert feed is distributed daily in very small quantities to accustom fish to a new flavour. To incentivate its consumption, it is advisable to start the distribution of inert feed in the morning, after the night starvation, and well before live feed is offered. Once dry food is accepted by most fish, it can be distributed by automatic feeders. To induce feed ingestion by the rest of the fish population, brine shrimp can be dropped close to the automatic feeder. The slow water movement will attract fish just below the feeder. When feeders are employed, distribution can be automatically set with a timer. In any case it is recommended to watch closely the ingestion rate. Artificial feed leftovers have to be avoided not only because they represent a loss of money, but mainly because they can severely pollute the water in the tank.

From a management point of view, feeding fish with an artificial diet involves a series of changes in the rearing environment (see Annex 13):

- aeration should be slightly increased to keep the inert feed in suspension;
- water renewal should be gradually increased to remove faeces and uneaten food particles, as well as to dilute ammonia and to supply more oxygen, which is related to an increased metabolic requirement;
- the frequency in cleaning the bottom of the tank should be increased to remove more debris and keep associated bacteria to a minimum.

In larval rearing cannibalism is not yet a major problem as it is in the weaning unit, but towards the end of the production cycle it may require attention, in particular when fish of different sizes and stage of metamorphosis coexist. The problem tends to be more serious with seabass. In presence of cannibalism, the supply of both artificial and live feeds should be better calibrated and its frequency should be increased. Another possible solution is to dilute the population in two or more tanks or introduce microalgae again, which would reduce the visibility and in consequence the more aggressive behaviour. This subject is extensively treated in the description of the weaning sector below.

Feeding protocol

As already indicated, the diet of seabass and gilthead seabream post-larvae in their first weeks of life is represented by small animals, rotifers and brine shrimp larval stages, whose biology, culture methods and nutritional value have been previously discussed in the manual. Microalgae are also provided to feed rotifers and improve the overall quality of the rearing environment. This section will deal with their handling and distribution to fish.

The feeding protocols for gilthead seabream and seabass are given in Annexes 17 and 18 respectively. In both cases, the protocol is based on the following assumptions:

- initial density of 150-200 post-larvae/litre;
- water temperature of 18°C;
- salinity of 35-37 ppt;
- photoperiod of 16 h light and 8 h darkness;
- all quantities mentioned are referred to a culture volume of 1 000 liters in order to adjust easily the feeding ration to tanks of different capacity;
- the feed quantities at different ages are only indicative as the actual rearing conditions change continuously from tank to tank.

Gilthead seabream aged 3 to 7 days receive a daily amount of 20 million rotifers per tank together with 40 liters of mature algal culture (at $12x10^6$ cell/ml). From day 8 to day 12 the amount of rotifers is increased by 20% to 24 millions and to 28 millions from day 13 to 16, whereas the algal supplement remains at 40 litres.

From day 17 the first brine shrimp nauplii are fed to the postlarvae: they should be of a particularly small strain in order to facilitate their gulping by the still small-mouthed fish. The Artemia AF cyst are an example of such strains. The amount used ranges from 0.1 to 0.5 millions. At the beginning, frequent controls on fish are recommended to check is they are accepting the new food item. Together with the first nauplii, the rotifer ration is increased to 32 millions, whereas microalgal supplements are progressively reduced to 20 litres. At this time the first artificial feed (of a very small size, 80-200 µm), is also distributed. The quantity offered is limited to 1-3 g, but its function at this stage is mainly to start getting postlarvae accustomed to this new taste.

From day 20, algae are further reduced to 10 litres, rotifers quantities begin to decrease (to 20 millions), being replaced by an increased amount of artemia AF (0.5-1 millions) and, for the first time, also artificially enriched artemia metanauplii, produced with cheaper artemia strains (0.3-0.6 millions are offered). Inert feed is also gradually increased to 10 g.

From day 24 to day 27 algae are progressively eliminated, rotifers decrease to 10 millions, whereas artemia AF increases to 1.5 millions and artemia EG or RH to 3 millions. Artificial feed is also increased to 15 g.

From day 28 the distribution of algae, rotifers and artemia AF nauplii is suspended and only artemia EG or RH (10 millions) and inert feed (15-20 g) are fed to fish, whose average weight should now be about 5 mg.

From day 34 to day 39 fish are given more EG or RH artemia (12 millions) and 20 g of inert feed of 80-200 µm size, plus 10 g of the larger 150-300 µm size.

From day 40 to day 43, when metamorphosis from post-larval to juvenile shape (fry) has started, the distribution of the 80-200 µm inert feed ceases, and it is replaced by more EG or RH artemia (up to 16 millions) and an additional 20 g of 150-300 µm feed. From this point the fish are ready to be moved to the weaning sector.

Daily distribution of live feed

Rotifers and brine shrimp are distributed by hand into the areas of the tank surface where larval density is lower. Feed is distributed three times per day, starting as soon as the lights have been switched on in the morning until four hours before the artificial sunset, in late evening. A quick distribution of the first ration in the morning is recommended to stop the forced starvation, which takes place during darkness.

The daily ration should be distributed every 6 hours in the following way (time is indicative):

- 50% at 08.00 h;
- 25% at 14.00 h;
- 25% at 20.00 h.

A prey density check is highly recommended before the second and the third distributions to adjust the concentration and thus avoid situations of over or under feeding. Use a 1-ml pipette to take two to three samples at different places in the tank and count the number of rotifers or artemia nauplii at naked eye or with the help of a portable lens.

From an operating point of view, the head of the larval sector prepares a feeding schedule for the day with the quantities to be distributed to each tank for the three meals. Rotifers and brine shrimp are quoted in millions: the worker has therefore to convert this figure in liters of stocked culture, according to the density marked on each stocking tank.

The live feeds should be distributed by keeping the jar above the water surface at the centre of the tank, and gently pouring its content over the rising air bubbles, to obtain an optimal dispersion. The operator has to take the highest care to avoid any splashing, waves or current the could induce stress to larvae.

First feeding with rotifers has already been discussed. Shifting from rotifers to brine shrimp is done progressively, feeding artemia nauplii from small size strains to gradually adapt the growing larva to the new food. The worker should check the ingestion of *Artemia* by regularly sampling larvae with a transparent beaker and looking at their digestive tract, which assumes a pale orange coloration when nauplii are ingested. See Annexes 21 and 22 for examples of record-keeping forms to help daily and weekly management of live food production and distribution.

Daily storage of live feed

As soon as produced, live feed is stored in the daily stocking tanks of the larval rearing unit, to be available for distribution during the hours when the lights are on. The most practical tanks are PRF rounded containers with a conical bottom equipped with a drainage ball valve. Their capacity ranges from 100 to 500 litres although larger tanks are also used, according to the larval unit size. A central strong aeration obtained with a coarse air stone maintains live feed in suspension and keeps oxygen levels within safe margins. It is in any case advisable to install an emergency supply of pure oxygen as not only the nutritional value of dead rotifers and brine shrimps is low, but also the risk that they can spoil the rearing environment is very high.

To prevent the loss of their nutritional value, live feed is kept at low temperatures (5-10°C) which reduce the metabolic rate. For this purpose, a complete heat insulation is applied to the tank sides, bottom and removable top. Styrofoam or polyurethane mats and bubble plastic foils are commonly used. Sealed ice bags or blue ice packs are then introduced in the tank and regularly replaced when melted. To avoid contamination, sealed ice bags should be discarded after use, whereas blue ice packs should be disinfected in a 500 ppm hypochlorite solution before reuse. Remember to control the presence of punctures and to thoroughly rinse them with freshwater before freezing.

An alternative and more appropriate option is to stock the concentrated cultures in buckets into a big freezer, whose thermostat has been set to a temperature range of 0-10 °C. The lid of the freezer is drilled to fit the air or pure oxygen hose and supply the highly dense populations with the necessary oxygenation and water circulation. As already mentioned the best cold storage is obtained using a refrigerated tank.

Maximum daily stocking density for live feed is as follows:

- rotifers: 10 millions/litre;
- artemia nauplii: 5 millions/litre;

Table 3.11- Daily schedule of controls

Hour	Feeding	Water control [1/]	System Control [2/]	Biological control	Various works
08.00	1	T	WAF	Rotifer/Artemia quality	Light switched on, Change screens
09.00				Larval behaviour	Clean make-up cartridges [3/] Clean UV/by-pass Siphon tank bottom
09.30		T-DO			
11.00				Larval behaviour	
11.30				Rotifer/Artemia quality	
12.00		T-DO			
12.30			WAF		
14.00	2	T-DO	WAF	Rotifer/Artemia quality	Siphon tank bottom
15.00				Feeding	
17.00				Rotifer/Artemia quality	
17.30		T-DO	WAF		
18.00				Larval behaviour	
20.00	3	T-DO	WAF	Larval behaviour	Change screens
24.00					Switch off light