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Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 47

2000
pages 61-73

To cite this article / Pour citer cet article

Carrillo M., Zanuy S., Oyen F., Cerdá J., Navas J.M., Ramos J. **Some criteria of the quality of the progeny as indicators of physiological broodstock fitness.** *Recent advances in Mediterranean aquaculture finfish species diversification.* Zaragoza : CIHEAM, 2000. p. 61-73 (Cahiers Options Méditerranéennes; n. 47)

CIHEAM - Options Mediterraneennes

Some criteria of the quality of the progeny as indicators of physiological broodstock fitness

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SUMMARY – The knowledge and control of the factors that affect maternal transport of nutrients to developing embryos, as well as the criteria for assessing the quality of the eggs produced and the broodstock fitness, are of paramount importance for the reproductive success of any particular species which are relevant for aquaculture. The factors that can act as determinants of the quality of the progeny as well as the possible indicators of both the quality of the eggs and the broodfish physiology are described and discussed. Emphasis has been paid to the role of highly unsaturated fatty acids (HUFA), which are enclosed into the formulated diets provided to broodfish, in the quality of the eggs and larvae obtained.

Key words: Egg quality determinants, broodstock fitness, maternal nutrient transport, teleost.

***RESUME –** "Quelques critères de qualité de la descendance comme indicateurs de l'adéquation physiologique des reproducteurs". La connaissance et le contrôle des facteurs qui concement le transport maternel des nutriments pour le développement des embryons, ainsi que les critères pour établir la qualité des œufs produits et l'adéquation des reproducteurs, ont une grande importance pour la réussite dans la reproduction de certaines espèces d'intérêt pour l'aquaculture. Y sont décrits et discutés les facteurs qui peuvent être décisifs pour la qualité de la descendance, ainsi que les possibles indicateurs de la qualité des œufs et de la physiologie des géniteurs. On a insisté sur le rôle des acides gras polyinsaturés (HUFA), qui sont présents dans la formule de composition des régimes des reproducteurs, ainsi que sur la qualité des œufs et des larves obtenus.*

***Mots-clés :** Qualité des œufs, adéquation des reproducteurs, transport maternel des nutriments, téléostéens.*

Introduction

Maternal transport of nutrients to developing embryos is a process of paramount importance for the survival of any teleost species either in wild or culture conditions. To understand this process and particularly their consequences, it is necessary to study several aspects placed between the reproductive physiology and nutritional fish physiology which require the tools developed in both disciplines. Morphological, physiological and biochemical characteristics of the resulting progeny are direct or indirect consequences of the previous nutrition of the broodfish during some time before the reproductive process can take place. Consequently, if the previous nutrition of broodfish is adequate, the resulting eggs can be of good quality and will have all the potential to produce viable fry and thus guarantee the survival of any newly cultivated species. Teleost fish is endowed with the ability to sense nutritional state and to regulate their reproductive development and activity accordingly. In general, inadequate nutrition retards growth and delays sexual maturation; high nutrition and rapid growth advance maturation. Neuroendocrine research on metabolic regulation of reproductive function is of fundamental importance to understand the mechanistic links between somatic metabolism and the neural/humoral control of reproduction. External indications of when the reproductive activity is initiated include gradual changes in behaviour and body appearance. These changes reflect a chain of events originating in the brain: increasing production of sex steroids by the gonads in response to the increasing secretions of gonadotropins from the pituitary gland, which in turn is being driven by the increased secretion of gonadotropic-releasing-hormone (GnRH) from the hypothalamus. The beginning of this cascade of hormones is determined by a variety of signals routed through the brain to the neuroendocrine mechanisms that control GnRH secretion. Some of these signals originate internally and relate to growth (i.e. metabolic signals) whereas others are external and provide information about the fish's environment (i.e. melatonin produced by the pineal gland as a consequence of photoperiod changes of the environment). In teleosts, during gametogenesis and in response to increasing steroids by the gonads, both free fatty acids and free amino acids are mobilized from the adipose tissue and muscle protein respectively and transferred via the serum to the liver where they are ensemble into the egg-specific lipoprotein, vitellogenin. This very high density lipoprotein (VHDL), consisting of approximately 80% proteins and 20% lipids, two-thirds of the lipid accounts for phospholipids and one-third for triacylglycerols, and because of the abundance of phospholipids, the lipid vitellogenin is rich in (n-3) highly unsaturated fatty acids (HUFA), especially docosahexaenoic acid (22:6n-3, DHA) (Sargent, 1995). During egg formation, vitellogenin is taken up by developing oocytes by a process of pinocytosis and is cleaved in the egg to generate the egg yolk proteins, lipovitellin and phosvitin, which are the components of the vitellus (Fig. 1). Therefore the bulk of the (n-3) HUFA-rich phospholipids in eggs is located in lipovitellin. Both phospholipids and triacylglycerol are catabolized to provide energy for the developing eggs and larvae, but the chief role of (n-3) HUFA is the formation of cellular membranes. Taking into account that neural cell membranes are very rich in 22:6n-3, and the high neurosomatic index in larval fish, this fatty acid has a critical role in the formation of brain and the eyes at these earlier stages. However the causative agents of the determinants of egg quality are numerous and these are not only reduced to formulation or the ratio of the diets provided to broodstocks although this constitutes the bases for the maternal influences of the quality of the progeny. Other factors such us culture conditions (water quality, stocking, diseases), environment (temperature, light, salinity), selection procedures (inbreeding, conservation), manipulation (netting, anesthetizing, transporting, injections, sampling), husbandry, techniques of spawning induction and sex control, over-ripening of eggs and stress in general would affect either directly or alter the transport of nutrients from the broodfish to the developing eggs. To complicate this picture even more, there is a lack of agreement regarding reliable methods for the assessment of quality of the eggs (Kjørsvick *et al.*, 1990), which is indispensable to evaluate the effects of the factors which determine egg and larvae quality. This aspect is very important because, poor quality eggs, generally produced as a consequence of an inadequate broodstock management, may decrease the survival potential of the hatched larvae and this will affect directly the production success of any fish farm. Consequently, it is an essential prerequisite to have reliable and simple methods for the assessment of egg quality, which in turn will provide valuable index to assess the physiological broodstock fitness under certain culture conditions. The reverse could also be true. It is indispensable to have a good diagnostic method of maturation, nutrition or fitness of broodfish under any circumstance, which will enable us to predict what will be the quality of the resulting progeny.

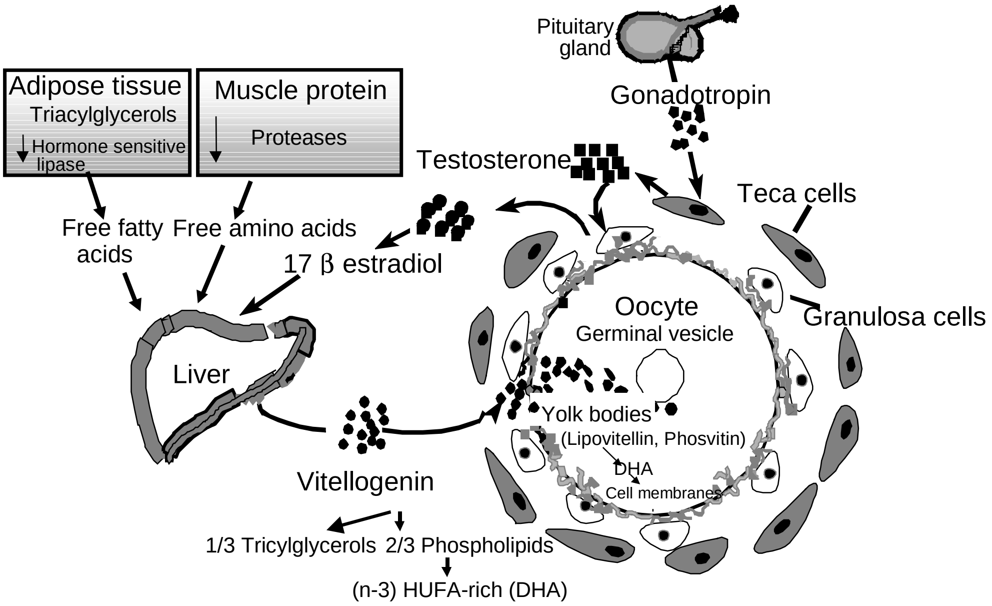

Fig. 1. Hormonal regulation of vitellogenesis in teleost fish. After Nagahama *et al.* (1995) and Sargent (1995).

Some characteristics which define the quality of the progeny

Several characteristics and methods have been proposed to define the quality of the progeny.

However none of them by itself is enough to fully characterize it, but a combination of them gives more satisfactory results. This is more evident if we consider that good quality eggs may exhibit low levels of mortality at fertilization, hatch and up to the first-feeding and particularly when they are expected to produce the healthiest and fastest growing fry. Besides not all of these characteristics behave in the same way in the different species of fish and they are not always consistent indicators of the quality of the progeny. Anyhow, and considering the use of them by different laboratories and hatcheries, we can list them as follows:

(i) Egg size (diameter of the egg).
(ii) Shape and transparency of the egg.
(iii) Buoyancy.
(iv) Number and distribution of the lipid droplets.
(v) Biochemical composition of the eggs [vitamins, carotenoid, lipids, fatty acids (particularly DHA), eicosapentaenoic acid (20:5n-3, EPA), araquidonic acid (20:4n-6, AA) and their relative proportions, others].
(vi) Rate of fertilization.
(vii) Appearance of the chorion.
(viii) Cell symmetry.
(ix) Chromosome appearance.
(x) Hatching rates.
(xi) Larval survival rates.
(xii) Morphological deformities.

To assess the quality of the eggs of any species of teleost it is a prerequisite to have a reliable incubation system by which the characteristics and survival of the developing eggs and embryos can easily be recorded. Any conventional incubation system includes eggs in a sieve, with recirculating, UV sterilization and heated water, and aeration. A known amount of fertilized eggs is placed in these units (typically several hundreds) and different quality indexes (see above) are recorded in the following stages of egg development. These indexes are generally expressed as percentages. Unfortunately this system has some limitations because it deals with populations on which manipulation and social factors reduce the rates of survival of progeny and changes in individual eggs are impossible to follow. Consequently, the results obtained, expressed as averages, are sometimes only estimates of a time consuming process of counting part of a population.

In order to improve the accuracy of egg and larval studies we developed a new system of incubation consisting of plastic micro titre plates with 96 wells on which one egg per well was allocated with 0.2 ml of sterilized water. The plates were covered with a lid and the total was transferred to a small transparent zip-lock bag in order to prevent evaporation. Incubation of the plates was performed in an incubation chamber at 16°C. The performance of this new system was compared with those of a conventional recirculating incubation system commonly used in most facilities (Fig. 2). Results indicate that after 8 days of incubation, mortality of the larvae on recirculation system was of 92.4 ± 3.31% while on the micro titer-plates system it attained only until 7.15 ± 1.6%. Besides, deformation rates (% of larvae showing a jaw deformation at ten days after fertilization) were reduced from 38% observed in the conventional system to 0.06% in the microtiter-plate system.

Other considerations regarding the quality of the progeny will be mentioned in detail further on.

Factors that influence the egg quality

Many factors have been suggested as possible determinants of the progeny quality (see above). However only few of them have been clearly shown to influence the progeny quality and can be listed as follows:

(i) Broodstock management.

- Genotype.
- Nutrition status of the broodfish.
- Spawning induction.
- Stress.

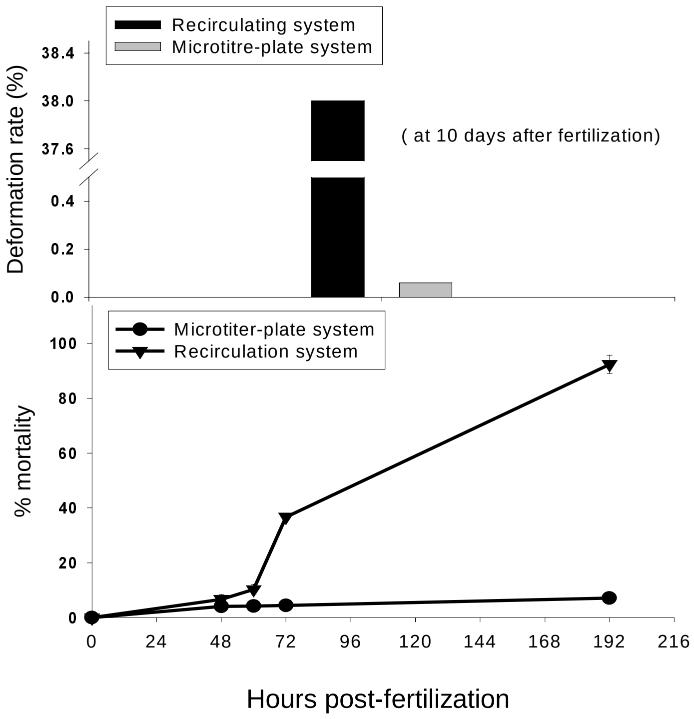

Fig. 2. Early mortality and deformation rates of sea bass reared in two incubation systems starting with good quality eggs obtained from a normal spawning. Bars represent means of 600 larvae. Symbols in the lines represent mean values of 96 eggs and vertical lines the standard error of the mean. Modified from Oyen *et al.* (1997).

(ii) Ageing processes.

- In adults:

- Time of the first spawning (puberty).
- Ageing of the broodfish (old animals).

- In eggs:

- Overripening.
- Bacterial colonization of the surfaces of the eggs.

Some of these will be considered in more detail further on.

Physiological indicators in the broodfish

Physiological indicators in fish could be numerous but these depend on the subject of study, i.e., disease, stress, reproduction, growth and nutrition. In this work we are only going to consider a few selected indicators of nutrition and reproduction of the broodfish which have likely been related to the egg quality and can be listed as follows:

(i) Morphological indexes.

- Weight (*W*) and size (*L*) (cumulative).
- Visceral-fat-somatic-index (*VFSI*): 100 visceral fat *W*/body *W*.

- Hepato-somatic-index (*HSI*): 100 liver *W*/body *W*.
- Instantaneous growth rate (*G*): 100 (ln W_2 – ln W_1)/(t_2 – t_1).
- Condition index (*CI*): 1000 body *W*/body L^3.
- Gonado-somatic-index (*GSI*): 100 gonad *W*/body *W*.

(ii) Reproductive and nutritional performance.

- Total or absolute fecundity (*TF*): no. eggs/fish (during all period of spawning).
- Relative fecundity (*RF*): no. eggs/kg post stripped fish.
- Spawning frequency (*SF*): no. of spawns/fish.
- Spawning spread (*SS*): no. of days on which spawns occurs.
- Time (*t*) of the beginning of the first spawn(t_{1s}): date of the first spawning.
- Daily food intake (*I*): 100 (average food intake/fish/day)/0.5 (W_2 + W_1).
- Food conversion (*FC*): 100 *G/I*.

(iii) Hormones and compounds.

- Gonadotropic-releasing-hormone (GnRH) contained in the brain and in the pituitary gland.
- Gonadotropin-II (GTH-II) levels in plasma.
- Sex esteroids (11-ketotestosterone, 11-KT; Testosterone, T; and 17 β-oestradiol, E_2) in plasma.
- Plasma insulin.
- Vitellogenin (VTG) in plasma.
- Plasma glucose.
- Lipids, fatty acids levels in plasma (particularly the ratios of DHA:EPA, AA:EPA, and n-3:n-6), others.

(iv) Histology.

- Gonads: qualitative and quantitative assessment of germinal cells.
- Liver: cell morphology and quantitative assessment of cells.

Selected samples of factors which affect fecundity and egg quality

Reproductive strategies, body and egg size

Oviparous fish produces a large number of eggs compared domestic animals and among fish species, there are also considerable differences between them. Most marine fish produce millions of eggs whilst other fresh water species only account for thousands. Moreover, most marine species of fish produce multiple batches of eggs at regular intervals during a few months per year whilst other species produce a single batch of eggs each year. These considerations with respect to the fecundity of the species are very important to planning and management of broodstock facilities, particularly due to the costly installations required for these less fecund species. In addition to fecundity, *egg size* is another parameter to be considered in assessing the egg production capability of the broodfish. It is generally accepted that in the way that a fish size increases so does fecundity and the diameter of the eggs produced. However in rainbow trout, a gradually diminishing increase in fecundity with increasing fish size (which means that larger broodfish have lower relative fecundity) has been reported (Bromage *et al.*, 1992). As has been quoted in Bromage (1995), 1 t of smaller broodfish trout would produce up to twice as many eggs as the same overall weight of individually-larger fish. These considerations are of great interest when assessing the fecundity of the new marine species for aquaculture in relation to the size and age of the broodfish and also with the size of the eggs produced, particularly if these are pelagic. As the eggs in most of the hatcheries are collected using a fine gauge nets placed across the outflow of the tanks those can be checked daily to know the spawning season or *SS* by observing the period between the first and the last spawning. The mean spawning time (*mst*) can also be calculated by using a value equal to the mean number of days passed between the first and successive spawns. Fecundity can be calculated in a practical way by placing the eggs obtained from a broodfish, in a measuring cylinder in order to determine the volume of both the floating (viable) and sinking (non-viable) eggs. As the egg size may vary from one spawning to another and according to the size of the broodfish, the number of eggs contained in a specific volume may also be different. Consequently it is a pre-requisite to previously calibrate the method by measuring the egg diameter and counting the number of eggs in a significant number of sub-samples collected from the spawns of different broodfish during the spawning season. In this way a typical regression equation (typical regression equations) for the number of eggs per unit of volume and the egg diameter can be obtained. For sea bass this equation is as follows:

$$N = 2865 - 1796\,D;\ P < 0.001,\ r^2 = 0.94$$

where N is the total number of eggs in 1 ml and D is the mean egg diameter of 100 eggs. This formula corresponds to the regression line obtained when, in 25 different spawnings, the mean diameter of 100 eggs was related to the number of eggs observed in 1 ml (Navas *et al.*, 1998). It would be desirable to calibrate the measurement of fecundity for each new species that can be considered for aquaculture. Once we have a reliable method for assessing the fecundity we can study the factors that affect the egg quality and get firm conclusions about their effects.

Broodstock genotype

In addition to fish size a further determinant of fecundity and egg size is the genotype of the broodfish. Unfortunately, no data is available for marine species and this will be addressed only to salmonid. Work with either commercial stocks or wild populations of trout showed that the most fecund stocks/strains produced twice as many eggs as the least fecund and a 10% difference between the smallest and largest eggs (Abée-Lund and Hindar, 1990; Bromage *et al.*, 1990). These studies give evidence of the existence of the wide possibilities of improving the productive efficiency of any farm by choosing strains for potential broodstock which can have profound effects on the number and sizes of the eggs produced.

Food ration

Another effect on fecundity and egg size could be the daily and seasonal rates of feeding of broodstock diets. It is known that food availability is one of the main factors affecting reproductive process in fish populations (Bye, 1990) and the effects of food ration on reproduction have been extensively studied in many fish species (bibliography in Cerdá *et al.*, 1994a,b). It is generally accepted that food restriction may reduce total fecundity, delay maturation and affect egg size, however it is not clear how food deprivation affects gonadal development and fecundity and how the operating mechanisms in these processes are. Feeding broodstock sea bass at half of their recommended daily ration of food throughout the year resulted both in a reduced specific growth rate in weight, lower CF, glucagon, insulin (during the vitellogenic period), glucose and estradiol plasma levels and in a delay of emergence of vitellogenic oocytes in the ovary (although with no significant changes in the plasma vitellogenin), a retard in the spawning time and a significant reduction in the size of eggs and larvae) (Cerdá, 1993; Cerdá *et al.*, 1994a). These data suggest that the E2 low circulating levels observed in fish fed with restricted rations could decrease the oocyte growth rates, delay the production of viable eggs (buoyant) and reduce the egg size. In general larger eggs produce larger fry and as the egg size was reduced in the restricted diet group, there was a tendency to increase the fecundity and the number of spawning per fish in this group. On the other hand and despite clear differences in size of the eggs and hatched fry (assesses at ten days) of the two groups, there were no reductions in viability expressed on the basis of mortality levels at hatching or after 40 days of culture. The absence of any reduction in viability of fry derived from small eggs also agrees with findings of many other authors (Springate and Bromage, 1985; Monteleone and Houde, 1990; Baynes *et al.*, 1993). We can conclude, that the changes observed in low-ration fed females did not seem to seriously affect the egg or larval survival in captivity, besides not any important differences in the biochemical composition of the eggs produced by the two groups was found (Cerdá *et al.*, 1994a,b) supporting the above statements. Despite the reduced size of the eggs produced by the fish with restricted ratios, the fecundity, number of spawns/fish and spawning spread were higher than the full diet group, indicating compensatory physiological behavioural reproductive mechanisms in sea bass.

Studies involving seasonal changes in ration in rainbow trout revealed the existence of "windows of opportunity" during which high and low feed rates produce significant effects on fecundity and rates of maturation (Bromage, 1995). Unfortunately these types of studies have not been performed in any marine fish species.

Food composition

Gross nutrient availability

Since the pioneer studies of Watanabe *et al.* (1984a,b,c,d) done with the red sea bream there have been increasing evidences that the composition of the diets fed to several species of broodfish during the pre-spawning period have a major impact on the quality of their eggs and larvae (see Bromage, 1995). Cerdá *et al.*, (1994b) and Kah *et al.*, (1994) found that in the sea bass feeding broodstock, two isocaloric diets, with different protein and carbohydrate contents, resulted in major differences in the growth, numbers, viability, quality of eggs and larvae deformities as well the basic mechanisms of the reproductive process. More specifically, in fish fed with a high protein-low carbohydrate diet (HP), fecundity, buoyancy and hatching rates were 1.5, 1.5 and 10 times higher than the group fed with low protein-high carbohydrate diet (LP) respectively. On the other hand, LP, presented increased levels of insulin in plasma and a reduced release of GnRH at the brain level during the spawning season. In addition, the levels of E_2 resulted also inhibited in this group (Fig. 3). These results provide an indication that the protein and/or carbohydrate composition of diets may affect the reproductive process at multiple sites and notably at the brain level and this in turn has important consequences on the quality of the eggs produced.

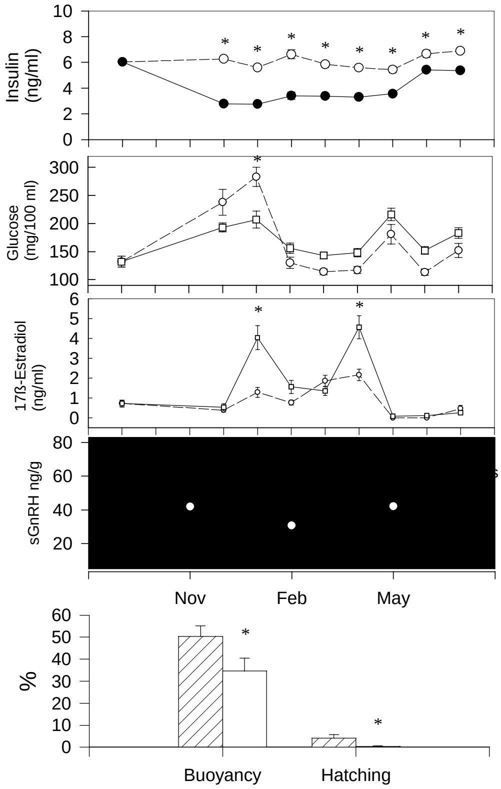

Fig. 3. Effects of two isocaloric diets with different protein and carbohydrate content on plasma insulin, glucose, 17 β-estradiol, pituitary sGnRH content of the sea broodfish and on the quality of the eggs produced in terms of buoyancy and hatching rates. Solid lines and hatched bars indicate high protein-low carbohydrate diets; stripped lines and open bars indicate low protein-high carbohydrate diets. Asterisks indicate significant ($P < 0.05$) differences between diets except for sGnRH which indicates monthly differences for the same group. Modified from Kah *et al.* (1994) and unpublished results.

Micro nutrients: Seasonal demand of essential fatty acids

Although gross nutrient availability is clearly important in influencing various aspects of reproductive physiology it is very difficult to attribute the affected quality of the eggs to individual components of the diets such as micro nutrients, i.e. amino acids, HUFA, vitamins and carotenoids. Today an increasing attention is being paid to the role of HUFA on the egg quality. It is well known that marine fish have high dietary requirements for HUFA of the n-3 series, principally, DHA and EPA in relation to the reproductive process (revised by March, 1993; Sargent, 1995). This requirement derives from a deficiency of Δ5-desaturase, an enzyme which is necessary for the formation of DHA and EPA from the C_{18} precursors (linoleic acid, 18:2n-6 and linolenic acid, 18:3n-3). Fernández-Palacios *et al.* (1995), reported that the spawning quality of gilthead sea bream was directly affected by an increased n-3 HUFA content of broodstock diets but only until a certain limit (1.6%), beyond that, dietary n-3 HUFA reduced fecundity and larval survival. These results fit in well with the claimed essential dietary needs of juvenile marine fish comprised between 0.5-1.7% of the dry weight as long-chain n-3 fatty acid (Sargent *et al.*, 1995). There is now increasing evidence about the importance of an adequate supply of essential fatty acids to obtain a high quality of the eggs produced, however this supply need not necessarily be continuos but should be adapted to the seasonal physiological requirements. It is generally accepted that fish present endogenous rhythms including growth and reproduction, which can in turn be trained by environmental cues. In addition, these rhythms are cyclic and some functions (i.e. enhanced food intake, fattening, growth, maturation, spawning, etc.) can only occur in specific periods of the year. Consequently it is likely that the specific nutritional requirements could also be more crucial in certain periods of the sexual cycle than in others. To test this hypothesis, Navas *et al.* (1997) fed broodfish with high essential fatty acid (H-EFA) diets, during different periods of the sexual cycle: pre-vitellogenesis, vitellogenesis, spawning, and all year or control, while a diet containing a low concentration of EFA (L-EFA) was supplied at all other times. More specifically, H-EFA diets contained to a 1.4-1.8 fold increase of either AA, EPA or DHA and 10 fold increase of the n-3:n-6 ratio over the corresponding L-EFA diets while L-EFA diet contained 7.3 fold increase of linoleic acid over the corresponding H-EFA diet. Results (Fig. 4) demonstrated that the fatty acid composition of the eggs from broodstock, which received the H-EFA either throughout the year or during the period of vitellogenesis had higher n-3 values (particularly DHA and EPA) and higher (n-3):(n-6) ratios than those obtained from the other groups receiving the H-EFA diet during spawning or previtellogenic periods.

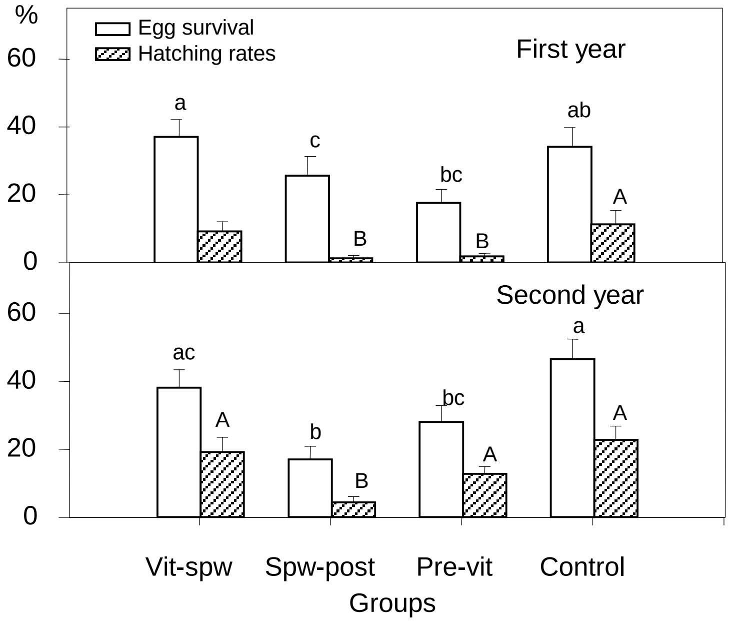

Fig. 4. Effects of high essential fatty acid diets supplied at specific periods of the year: vitellogenesis-spawning (vit-spw, Sept.-Feb.), spw-post spw (Feb.-Apr.), pre-vit (Apr.-Sept.), control (all year) while low essential fatty acids were provided at other times, on egg quality and hatching rates. Values represent the means of batches sampled from each diet group during the spawning season (± SE), suffixed letters in lower case for % viability and upper case for total survival to hatching. Columns labelled with different letters indicate the presence of a significant difference ($P < 0.05$). Modified from Navas *et al.* *(*1997).

These changes were correlated with the higher numbers of viable eggs at spawning and hatching rates in the control and vitellogenic group.

These results indicate once more the close links between the content of n-3 poly-unsaturated fatty acids (PUFA), especially the amounts of EPA and DHA, and egg quality. In addition, the vitellogenic period appears to be the most influential time during which these important HUFA, especially DHA, are incorporated more effectively into developing oocyte in a similar way as those broodfish which received H-EFA all the year. These types of studies could have very important economical applications in aquaculture because knowing the sensible period for requirement of EFA it will not be necessary to administrate them throughout the year to get similar results on egg quality. In this way the supply of H-EFA and expensive diets can be notably restricted only to a few months of the year with a great economic impact and efficiency.

Optimal ratio of some essential fatty acids

The limited ability of marine fish to convert EPA to DHA (Mourente and Tocher, 1993), illustrates the difficulty in establishing the requirements of marine fish for these important fatty acids. It is not only important to know the absolute amount of these fatty acids, but also the optimal dietary ratio of DHA:EPA, and (n-3):(n-6) ratio required to supply sufficient DHA to satisfy these requirements. Consequently both the diets provided to broodfish as well as the produced eggs should contain the correct balance DHA/EPA to ensure proper larval development on hatching. For a long time commercial diets for marine broodstocks were not properly formulated and results on the quality of the eggs and larvae obtained were very poor. A series of studies done by Cerdá *et al.* (1995, 1997) show the important deficiencies in HUFA which presented the commercial diets with respect to the natural diets (trash fish) particularly on (n-3):(n-6) ratio (about 2.5 vs 7.5 respectively) and DHA:EPA ratio (<1 vs 3.8 respectively). A prolonged administration of these diets (two years) had very important effects both on the physiology of broodfish as on the quality of spawnings of both sexes. In females it provoked an altered seasonal pattern of the plasma levels of E_2 and T with an increase in the early ovarian atresia which in turn provoked a reduced fecundity, spawning frequency, spawning spread, buoyant eggs, hatching rates and larval survival. In males some delays in the surge of testosterone at the beginning of the spermiation period and a dramatic decrease of the 11-KT levels throughout the entire period were observed. In parallel with these alterations the number of spermiating males were significantly reduced. These findings supported the hypothesis that the role of 11-KT might be related to the initiation of spermiation in sea bass and the depressed testicular steroideogenesis induced by deficiencies in dietary lipids may delay the spermatogenetic development affecting the timing of spermiation.

Bell *et al.* (1997) has recognized that AA is equally important in determining the quality of the eggs as this provides the basic materials for the formation of prostaglandins important in final maturation and must also be supplied pre-formed in the diet. Broodstock fish fed with trash fish (wet diets) generally produces higher quality eggs than fish fed with pelleted food containing fish and corn oil. Although DHA concentrations were similar for both diets, pelleted feed contained 1.3 mg/g of AA and an AA/EPA ratio of 0.1 while the wet diet contains 4.8 mg/g AA and AA:EPA ratio of 0.7. The fatty acid composition of the diets were also reflected on the eggs. The fatty acid composition of the eggs of fish fed trash fish contained significantly more AA, EPA and DHA compared to fish fed with pelleted food. AA is the major eicosanoid precursor in fish cells but if there is an excess of EPA over DHA, EPA has the ability to inhibit competitively the production and efficiency of eicosanoids derived from AA (Lands, 1989). Consequently broodstock dietary regimes should include increased ratios of DHA/EPA and AA/EPA for the benefit of improved quality and survivability of fish larvae.

The use of "wet diets" has been the most effective way to satisfy the EFA requirements of marine broodfish, however this practice could involve some risk of disease introduction and difficulties for storage. On the other hand and despite many commercial interests, which have expressed their desire to use an artificially fabricated pelleted diet, this has proved difficult, particularly with respect to their HUFA composition. Recently, Bruce *et al.* (1999) obtained an artificially fabricated diet for marine broodstock, optimizing the EFA composition, making use of a by-product of the Japanese tuna fishing industry, tuna orbital oil, very rich in EFA (ROPUFA diet). This diet allowed the manipulation of n-3 and n-6 HUFA in the resulting eggs, specifically AA, EPA and DHA with improvements in early survival and hatching success comparable to the reproductive performance obtained by the wet diets.

Consequently this artificial diet containing higher AA, DHA, EPA, AA:EPA, DHA:EPA than the artificial standard control diets, allows a production of high quality eggs.

Spawning induction

Environmental and hormonal manipulation for spawning induction in fish has been widely applied in several teleosts fish to alter the spawning time and get continuos supply of eggs and larvae to cover the ever increasing demands from growing fish farms. Unfortunately the studies on the effects of hormonal, photoperiod and thermoperiod regimes on the egg quality are very scan. A long term experiment of the effects of photoperiod regimes on reproductive performance and quality of the eggs of sea bass (Carrillo *et al.*, 1991, 1995) revealed that the reproductive events can be trained by photoperiod signals (i.e. exposure of long days with a month of duration, earlier or later in the sexual cycle, in an otherwise constantly short photoperiod regime). If these photoperiod signals are given earlier in the sexual cycle they may advance spawning time of the broodfish but when they are applied later in the sexual cycle they may delay them. However, the observed start of spawnings was not always consistently related to the onset of long or short day lengths. The observed spawning time did not coincide with the expected spawning time and this difference increased when earlier in the sexual cycle the photoperiod signal was applied. Quality of the spawning resulted also affected by this treatment and this was particularly evident in the more advanced or delayed induced spawnings, when a mismatch between the photoperiod and temperature regimes was produced by an incomplete environmental manipulation (i.e. only the photoperiod and not the temperature was manipulated). In addition, Zanuy *et al.* (1995) and Prat *et al.* (1999), reported that broodfish exposed to constant long photoperiod regimes induced a significant delay in the spawning time. The results indicated that the biochemical composition of the eggs was altered, as well as the fecundity, the percentage of buoyancy, the hatching and survival of larvae rates which were significantly lower than those of the group exposed to natural photoperiod regimes. This is a very interesting field of research. Unfortunately there is a big gap in the knowledge of the mechanism of action of either photoperiod or temperature at the brain level and how these signals are transduced into the neuro-endocrine system to get a successfully reproductive performance of the broodfish and obtain a good quality progeny.

Broodstock age

Effects of puberty

In fish, as in other vertebrates, the first transition from an infertile to fertile state (puberty) could be tightly coupled to some developmental changes in metabolic state in response to changes in nutrient partitioning as the animal grows. Once growth is sufficient (as reflected by appropriate changes in energy balance), the reproductive system will become active. It is generally accepted that the brain could detect this energetic balance through metabolites or metabolic hormones to signal high-level GnRH secretion and thus initiate the hormonal cascade to initiate the reproductive process. Unfortunately the metabolic signals that could serve as the molecular links between growth and reproduction remains elusive. In addition, it is necessary that during the early development of a fish, the neural connections and detectors should be completely formed before responding to any metabolic signals or external signals relating to the time of the year, which is important for puberty. timing. It is obvious that setting up the reproductive process for the first time in the life of any species would require a considerable effort of the organism to succeed in a full maturation of the brain-pituitary-gonad axis and with all the appropriate metabolic processes to be right on time. Consequently the broodfish during the first maturation and spawning, is expected not to produce all the eggs that it will usually produce in successive sexual cycles. This has been confirmed in sea bass on which both *TF*, *RF*, *SF* and *SS* increase several times more during the second sexual cycle than during the time of puberty (Fig. 5). There is some controversy with respect to the quality of the eggs generated during puberty, although it has been claimed that a tendency to improve the eggs and larvae’s quality in further spawns, corresponds to the next spawning cycle.

Effects of aging

Aging of the broodfish could also be determinant of egg quality. The fundamental mechanisms, which underlie ageing process, are generally poorly understood. One of the conspicuous features of the ageing is a decrease in plasticity expressed as a diminished capacity to compensate functionally for brain damage. In general, older females present an irregular and unbalanced process of gonadal maturation provoked by an important disruption of brain-pituitary-gonadal axis physiology linked to ageing process. In addition older fish tend to have an extraordinary large gonads which makes it more difficult to exert an harmonious control of their reproductive function. The eggs produced by an older female are of very poor quality in terms of buoyancy and hatching rates and with aberrant embryo developments as well. As a practical rule, at least for sea bass, it is very convenient not to utilize the eggs from either pubertal or from ageing fish but those pertaining to spawning collected between the second to fifth sexual cycle instead. This procedure will ensure a provision of eggs of good quality avoiding manipulation of very young or of oldest and larger broodfish. In both cases the broodstock management will reduce the cost of installations and sampling time, both of importance to optimise the yields of a fish farm.

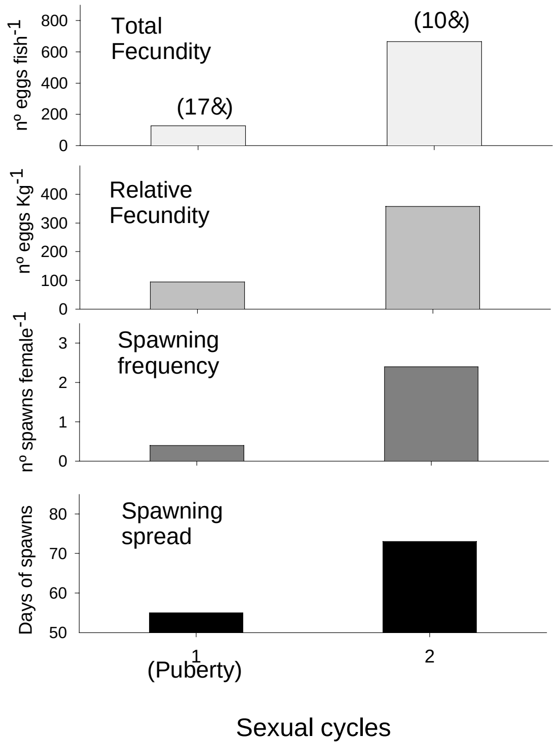

Fig. 5. Spawning performance of sea bass either during the first spawning (puberty) and during the second spawning period accounted from groups of 10-17 females placed with males in a sex ratio of 1(female):2(male). Modified from Cerdá *et al.* (1995).

Overripening

In nature, the reproductive events of any teleost fish are adapted and synchronized with the cyclic changes of the environment and in general spawns occurs spontaneously and the quality of spawns tend to be the best possible. However in culture conditions the animal's reproductive process can be seriously affected, resulting in an altered spawning performance with a very poor quality of eggs produced. Although some species can cope with the artificial environment of fish farms and may present spontaneous spawns with an acceptable quality of the eggs, most of them cannot reproduce spontaneously and fish have to be stripped and the eggs artificially fertilized. Choosing the time when any fish should be stripped is a crucial factor for the quality of the eggs obtained. After ovulation the unfertilized eggs are retained in the ovary cavity before being spawned. During this time the eggs undergo a series of morphological and biochemical changes to culminate into a period of optimum ripeness (maxima viability) after that, a process of aging known as overripening occurs, which is characterized by a progressive loss in quality. This loss in quality occurs when the eggs are retained in the body or stored in vitro in ovarian fluid and which the process are temperature dependent. The time after ovulation during which optimum egg quality is achieved varies with the different species and can last from an hour to several days (Kjørsvik *et al.*, 1990; Bromage, 1995). Improving our understanding of the process of overripening together with the ability to time ovulation and the period of optimum ripeness, should enable major improvements in the supply of significant amounts of good quality eggs to make the control of reproduction of any new species for aquaculture successful. We can conclude that the setting up the methods for assessing the quality of the eggs for a particular new species and identifying the factors which clearly influence the quality of the progeny are valuable tools for studies on the species diversification and to increase the variability and the yield of any fish farm.

Acknowledgements

This work were supported by an EC grant (AIR-CT93-1005), a grant from the Spanish Interministerial Commission of Science and Technology (CICYT; MAR96-1859) to M.C.

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