The kinetics of nutrient incorporation into body tissues of gilthead seabream (*Sparus aurata*) females and the subsequent effects on egg composition and egg quality

BY MOTI HAREL, AMOS TANDLER AND GEORGE W. KISSIL

National Center for Mariculture, P.O. Box 1212, Eilat, Israel

AND SHALOM W. APPLEBAUM

The Hebrew University of Jerusalem, Faculty of Agriculture, P.O. Box 12, Rehovot 76100, Israel

(*Received 14 July 1993 – Revised 14 October 1993 – Accepted 2 November 1993*)

The interaction between essential dietary components and changes in tissue nutrient reserves, egg quality and egg composition, were studied from 60 d before and during the spawning of *Sparus aurata* broodstock. Fish were given isonitrogenous (550 g/kg dry weight) and isolipidic (100 g/kg dry weight) diets, based on protein and lipid extracts of squid meal. Diets differed in the levels of *n*-6 (10–30 mg/g dry weight) and *n*-3 (0–10 mg/g dry weight) essential fatty acids. The effects of these diets on biochemical and fatty acid composition of body tissues, and the subsequent effects on egg composition and egg viability were measured. Dietary essential fatty acids were mostly incorporated into the liver, ovaries, digestive tract and associated adipose tissues. The lipid composition of these tissues reached an equilibrium with dietary lipid composition within 15 d of feeding on any given diet. Muscle and gill cartilage tissues did not show any significant changes in their biochemical and fatty acid composition, even after 60 d feeding. Egg viability decreased significantly within 10 d of feeding the broodstock with a diet deficient in *n*-3 highly unsaturated fatty acids (*n*-3 HUFA). The levels of *n*-3 HUFA in both polar and neutral fractions of egg lipid were directly correlated with their levels in the broodstock diet. When the total amount of egg *n*-3 HUFA dropped below 17 mg/g dry weight, egg viability and larvae hatching rate decreased by 53% and 47% respectively. These results suggest that the biochemical composition of organs involved in *S. aurata* reproduction are highly sensitive to the nutritional value of the diet, which affects egg and larval quality rapidly.

Egg composition: Egg quality: *n*-3 fatty acids: *Sparus aurata*

Many fish species tend to decrease their food intake during sexual maturation, and the energy and nutrients needed for ovarian growth are taken from their body reserves. Tissue preference from which the reserves are mobilized is species specific; female trout (*Salmo gairdneri* R.) mainly mobilize carcass and visceral lipid reserves (Nassour & Leger, 1989), freshwater catfish (*Clarias batrachus*) use abdominal fat as the main energy source for sexual maturation (Lal & Singh, 1987), while farmed Atlantic salmon (*Salmo salar*) use both muscle lipid and protein during sexual maturation, with a resulting increase in body water content (Aksnes *et al.* 1986).

Gilthead seabream (*Sparus aurata*) females continue to eat during sexual maturation and throughout the spawning season, and produce an egg biomass greater than their own body weight. In these circumstances material deposited in the ovaries must originate from the broodstock diet as well as from endogenous storage (carcass, liver and digestive tract), but the extent to which dietary or tissue reserves are utilized for ovarian growth, and how the diet quality affects the reproductive performance of this fish, is not known.

Studies on the nutritional effect on reproduction in common carp (*Cyprinus carpio*), rainbow trout (*Salmo gairdneri*) and red seabream (*Pagrus major*) showed that deficiency of *n*-3 highly unsaturated fatty acids (*n*-3 HUFA) in the broodstock diet reduces fecundity, fertilization, hatching rate, and viability of offspring (Shimma *et al.* 1977; Watanabe *et al.* 1984*e*, 1985*a*, *b*). The broodstock dietary effect on tissue and egg composition was demonstrated by Watanabe *et al.* (1984*d*), Mourente & Odriozola (1990*a*, *b*) and Washburn *et al.* (1990). They showed that the composition of essential fatty acids in eggs reflects the composition of the broodstock diet. However, the level of *n*-3 HUFA that eggs should contain in order to ensure an improved quality has not yet been established.

The objectives of the present study were to determine the interaction between essential dietary components and changes in tissue nutrient reserves of *S. aurata* females, and the subsequent effects on egg composition and egg quality.

MATERIALS AND METHODS

Diets

Squid meal of Indian origin was chosen as the source of protein and lipid for the experimental diets, after preliminary experiments demonstrated that it supports high fecundity and egg quality in gilthead seabream (M. Harel, unpublished results). The lipid fraction was extracted three times from the meal, using five volumes of a chloroform–methanol mixture (1:1, v/v), in addition to 0·1 mg/ml BHT (butylated hydroxytoluene, Sigma, Poole, Dorset). The pooled extracts were then washed with 0·8 g/l KCl according to the procedure of Folch *et al.* (1957) and the washed chloroform phase was evaporated to dryness in a rotary evaporator (40°). The non-lipid fraction of the squid meal was oven dried (40°) to remove trace organic solvents.

Three diets were formulated, using the non-lipid fraction of the squid meal as the protein source and the extracted squid oil and commercial soya-bean oil as lipid sources. Diet 1 contained full squid-oil extract, diet 2 contained equal amounts of squid and soya-bean oils, and diet 3 contained only soya-bean oil. The extracted squid oil was rich in *n*-3 HUFA while the commercial soya-bean oil was rich in *n*-6 polyunsaturated fatty acids (mainly 18:2 *n*-6). The biochemical composition and fatty acid content of these diets are given in Tables 1 and 2.

Broodstock

Two- and three-year-old fish were reared on a pelleted commercial diet (Matmor Feed-mill Corporation, Ashkelon, Israel), in circular 20 m^3 outdoor concrete tanks, receiving ambient sea water from the Gulf of Eilat. At 2 months before the spawning season, healthy fish (450 ± 100 g) were biopsied to determine their sex by removal of gonadal tissue using a micro-haematocrit capillary tube inserted into the gonad through the genital duct. These fish were used to stock six 600 litre experimental tanks randomly with eight females and eight males in each. The tanks were equipped with a standpipe drain and exposed to natural photoperiod. Water flow rate was sufficient to maintain the level of dissolved oxygen above 6 mg/l. Each of the three experimental diets was randomly assigned to two tanks. Fish were given a total ration of 15 g/kg body-weight per d, starting 2 months before spawning and continuing throughout the 4-month spawning season.

At 2 weeks after the shortest day of the year, December 21, twelve fertile males (determined by stripping) and six females (oocytes diameter > 400 μm) were selected from each of the three dietary treatments, and redistributed into six 600 litre tanks so as to contain one female and two males per tank. All the females in the experiment were injected intramusculary with 150 μl/kg body weight of mammalian gonadotropin-releasing-

Table 1. *Composition (g/kg) of the broodstock diets*

	Diets		
	1	2	3
Ingredients			
Squid meal (non-lipid fraction)	620	620	620
Wheat flour	235	235	235
Squid oil (extracted)	100	50	0
Soya-bean oil	0	50	100
Dicalcium phosphate	20	20	20
Choline chloride (60%)	5	5	5
Vitamin premix*	5	5	5
Trace mineral premix*	10	10	10
Methionine	3	3	3
Vitamin E	2	2	2
Ascorbic acid	1	1	1
Composition†			
Protein	537 (SE 17)		
Lipid	109 (SE 3)		
Ash	82 (SE 6)		
Moisture	51 (SE 12)		

* Vitamin and mineral premix, made by Koffolk Chemical Manufacturers, Petach, Tikva, Israel.
† Values given for the composition are mean values with their standard errors of the three diets, determined by Association of Official Analytical Chemists (1980) methods.

Table 2. *Fatty acid composition (mg/g dry weight) of the broodstock diets*

	Diet		
Fatty acid	1	2	3
14:0	0·7	0	0
16:0	8·9	7·3	7·6
18:0	2·8	2·4	2·4
18:1*n*-7	0·9	0·9	1·3
18:1*n*-9	6·5	10·1	18·6
18:2*n*-6	9·3	15·7	29·3
18:3*n*-3	1·4	2·4	4·7
20:1*n*-9	0·8	0·6	0
20:3*n*-3	1·5	0·9	0
20:5*n*-3	2	1·1	0
22:6*n*-3	5·5	3·1	0
Total saturated	12·4	9·7	10
Total monounsaturated	8·2	11·6	19·9
Total *n*-6	9·3	15·7	29·3
Total *n*-3	10·4	7·5	4·7
n-6:*n*-3	0·89	2·09	6·23

hormone analogue ([des-GLY^{10}-D-TRP^{6}]-leuteinizing-hormone-releasing hormone; Bachem Feinchmikalien AG, Switzerland; Gothilf, 1990).

In order to study the kinetics of the response of body tissues and egg composition to dietary changes, the diets were switched 1 month before the end of the spawning season,

so that fish receiving diet 1 which was high in *n*-3 HUFA were given diet 3 which had no *n*-3 HUFA, and vice versa. Fish receiving diet 2 continued to feed on the same diet, and served as a control group during the experimental period.

Eggs

Eggs were collected daily in a net basket connected to the overflow pipe of each spawning tank. Viable buoyant eggs were separated from the dead sinking eggs, and the total biomass of both fractions was determined. One hundred viable eggs from each spawning tank were incubated in 1-litre beakers supplied with a gentle flow of sea water of $19 \pm 0{\cdot}5°$. The percentage of normal hatched larvae and their survival up to 3 d after hatching was determined by counting under a microscope.

Tissue samples

Six females from each dietary treatment were sampled at each of the following times: 2 months before spawning, at first spawning, and at the end of the spawning season. Fish were killed and samples were taken of liver, digestive tract and associated adipose tissues, gonads, white muscle, and cartilage from the gill arches. Samples were washed with distilled water, weighed, and immediately frozen in liquid N_2. Tissues were subsequently lyophilized for 48 h, and kept at $-70°$ until analysed for biochemical and fatty acid composition. A sample of viable buoyant eggs from each spawning tank was taken daily, washed with distilled water and kept at $-70°$ for later analysis.

Analytical procedures

Dry matter, lipid and ash determinations of lyophilized tissue samples, eggs, and the experimental diets were made following Association of Official Analytical Chemists (1980) methods. Protein was measured according to the method of Lowry *et al.* (1951), using bovine serum albumin (Sigma) as a standard. Lipid extracts of the eggs were separated into polar and neutral fractions using silicic column chromatography (Christie, 1982). The fatty acids in the lipid extracts of body tissues, experimental diets and both polar and neutral fractions of egg lipid were transesterified with BF_3 in methanol (120 g/l; Sigma) according to the procedure of Morrison & Smith (1964), using heptadecanoic acid (17:0; Sigma) as an internal standard. The esterified fatty acid methyl esters were then extracted with hexane, and analysed using a Hewlett Packard 5890 gas chromatograph, equipped with a 30 m × 0·25 mm (i.d.) fused silica capillary column, 0·2 mm film thickness (Supelco, Saffron Walden, Essex) and He as carrier gas. Identification of individual fatty acids was carried out by comparison with known standards.

Statistical analyses

The experiments were conducted using a completely randomized block design. The spawning tanks were grouped into two blocks in the experimental design, since the experimental system included two different shapes of tanks. Analysis of variance (General Linear Model GLM ANOVA), provided by the Statistical Analysis System software (SAS Institute Inc. 1985) was used to determine the effect of diet, and tank within diet treatment, on each variable measured. Body weight was added to the model as a co-variate in the analysis of hepato-somatic index (liver weight × 100/(body weight − liver weight)), gonado-somatic index (gonad weight × 100/(body weight − gonad weight)) and fecundity. Percentage data were transformed into the arcsine square root for statistical analysis. In all the

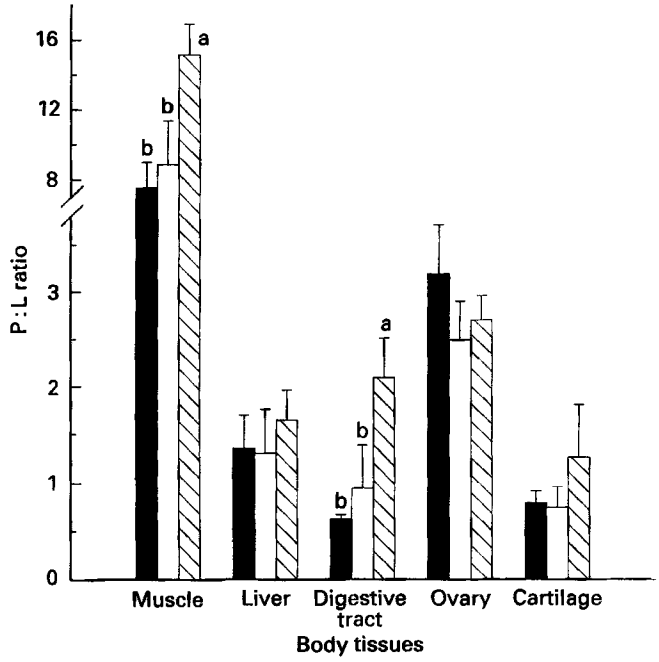

Fig. 1. Changes in protein:lipid (P:L) ratio in muscle, liver, digestive tract, ovary and gill cartilage tissues of *Sparus aurata* females during the reproductive season (■, 60 d before spawning; □, beginning of spawning, ▨, end of spawning season). Bars represent the mean values with their standard errors (n 6). Bars having different letters are significantly different from each other ($P < 0{\cdot}05$).

comparisons, homogeneity of variances was assessed by F_{max} ratio (Hartley's F_{max} test; Fryer, 1966). When significant differences ($P < 0{\cdot}05$) were found, means were then compared using Student's-Neuman-Keuls (SNK) multiple range test (Sokal & Rholf, 1981). The relationships between the tissues and egg fatty acid composition and broodstock dietary composition were evaluated by performing first- or second-order linear regression analysis, using the least squares fit method by Sigmaplot 5·0 software package (Jandel Scientific, San Rafael, CA, USA).

RESULTS

Changes in body weight and tissue biochemical composition were independent of dietary treatments. Body weight of females did not increase significantly with the approach of the spawning season, but by the end of the season all females, independent of dietary treatments, lost an average of 13·13 (SE 2·3)% of their initial body weight.

Ovarian development started 2 months before spawning. During this period the gonado-somatic index increased and reached a maximum of 4·37 (SE 1·93) by the onset of spawning, and the hepato-somatic index increased significantly ($P < 0{\cdot}05$) from 1·38 (SE 0·16) to 1·61 (SE 0·24). The increase in liver weight occurred without any significant change ($P > 0{\cdot}05$) in liver protein:lipid (P:L) ratio (Fig. 1). At the end of the spawning season, muscle and digestive tract tissues increased significantly ($P < 0{\cdot}05$) in their P:L ratio. This resulted mainly from a major loss of lipid, which was as high as 52% in muscle tissue and up to 45% in digestive tract tissue, regardless of the dietary treatments. The ash content in the gill cartilage tissue and liver increased significantly ($P < 0{\cdot}05$) by 45% and 52% respectively during the spawning season.

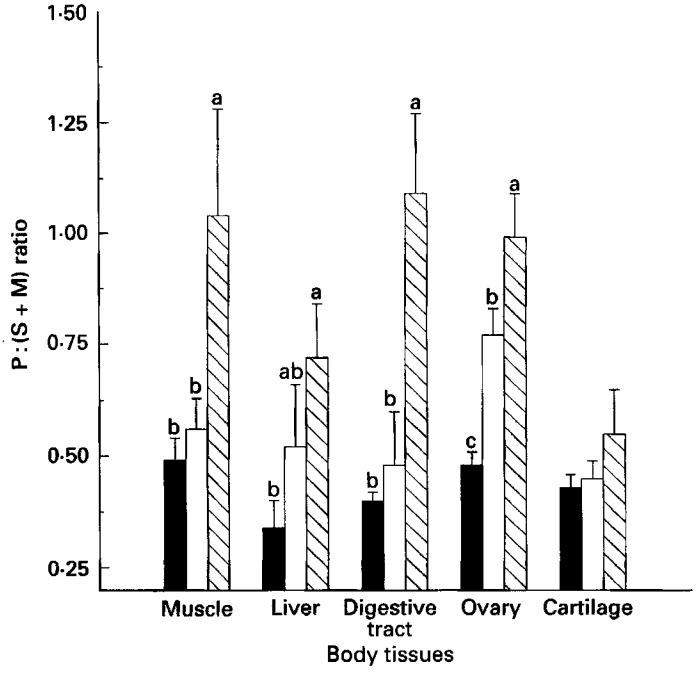

Fig. 2. Changes in the polyunsaturated:saturated plus monounsaturated fatty acid (P:(S+M)) ratio in muscle, liver, digestive tract, ovary and gill cartilage tissues of *Sparus aurata* female during the reproductive season (■, 60 d before spawning; □, beginning of spawning; ▨, end of spawning season). Bars represent the mean values with their standard errors (n 6). Bars having different letters are significantly different from each other ($P < 0{\cdot}05$).

At 2 months before spawning and at the onset of spawning the lipid in all body tissues was composed of 70·1 (SE 3·01)% saturated and monounsaturated fatty acids and 29·9 (SE 3·08)% polyunsaturated fatty acids ($\geq$ 18:2), independent of the dietary treatments. However, at the end of the spawning season, muscle and digestive tract tissues lost 50% of their saturated and monounsaturated fatty acids. This was reflected in the significant increase ($P < 0{\cdot}05$) in the polyunsaturated:saturated plus monounsaturated fatty acid (P:(S+M)) ratio in muscle and digestive tract tissues at the end of the spawning season (Fig. 2), regardless of the dietary treatments.

While no dietary effects were observed on P:(S+M) ratio of body tissues, the polyunsaturated fatty acid composition was affected by the dietary treatments. During the first 60 d feeding on any given diet, the polyunsaturated fatty acid composition represented by the n-6:n-3 fatty acid ratio in muscle and gill cartilage tissues was almost independent of the dietary composition (Fig. 3). The most notable changes in polyunsaturated fatty acid composition in response to dietary change occurred in the ovary, liver and digestive tract and their associated adipose tissues. When the diet was changed during spawning from a low n-6:n-3 fatty acid ratio (0·9) to a high ratio (6·28) or vice versa, ovary, liver, and digestive tract responded rapidly by changing their n-6:n-3 fatty acid ratio to match that of the diet. The kinetics of lipid deposition in these tissues indicate a high lipid turnover, which reached an equilibrium with the dietary composition within 15 d of feeding on any given diet. The equilibrium composition was determined as the composition of these tissues in fish given the alternative diet for 60 d.

As a result of the high lipid turnover in organs associated with reproduction (ovary, liver and digestive tract tissues), the essential fatty acid (18:2n-6, 20:5n-3 and 22:6n-3)

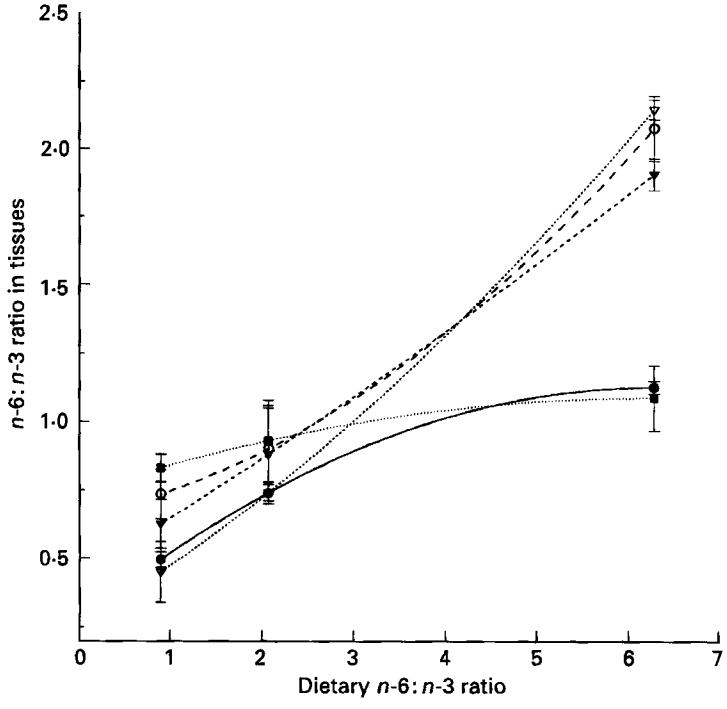

Fig. 3. *n*-6:*n*-3 fatty acid ratio in muscle (●), liver (○), digestive tract (▼), ovary (▽) and gill cartilage tissue (■) of *Sparus aurata* females fed for 60 d on diets which vary in their *n*-6:*n*-3 fatty acid ratio (0·9, 2·07, 6·28). The best fit curvilinear lines drawn between the points were determined by second-order linear regression analysis of the data. Each data point represents the mean values with their standard errors for six fish.

composition of the eggs responded quickly to the broodstock dietary change (Fig. 4). It also reached an equilibrium with the broodstock diet composition within 15 d following the change from diet 1 which was high in *n*-3 and low in *n*-6 fatty acids to diet 3 which was low in *n*-3 and high in *n*-6 fatty acids and *vice versa*. The equilibrium composition was determined as the composition of eggs from fish given the alternative diet for 60 d.

The effect of the broodstock dietary levels of essential fatty acids on the essential fatty acid composition of the eggs was equal for both neutral and polar fractions of egg lipid (Fig. 5). Linoleic acid (18:2*n*-6) was consistently higher in the neutral fraction of egg lipid, while both 20:5*n*-3 and 22:6*n*-3 were consistently higher in the polar fraction, regardless of broodstock dietary levels.

The preferential incorporation of essential fatty acids into egg polar or neutral lipids was tested by performing a series of first-order linear regression analyses between the content of each essential fatty acid in egg lipids and their corresponding content in the broodstock diet. The regression equations of these correlations are shown in Table 3. Comparison of the slopes (*a*) of these regression lines shows that 18:2*n*-6 was incorporated more easily into the neutral than the polar fraction of egg lipid, while 20:5*n*-3 and 22:6*n*-3 were incorporated at a similar rate into both neutral and polar fractions of egg lipid, in response to a dietary increase in these fatty acids. However, the rate of increase in egg 22:6*n*-3 was 70% higher than that of 20:5*n*-3 and double that of 18:2*n*-6. Fig. 5 also shows that a low level (9·2 mg/g dry weight) of *n*-3 HUFA was found in the eggs, even when *n*-3 HUFA was totally excluded from the broodstock diet for 60 d feeding.

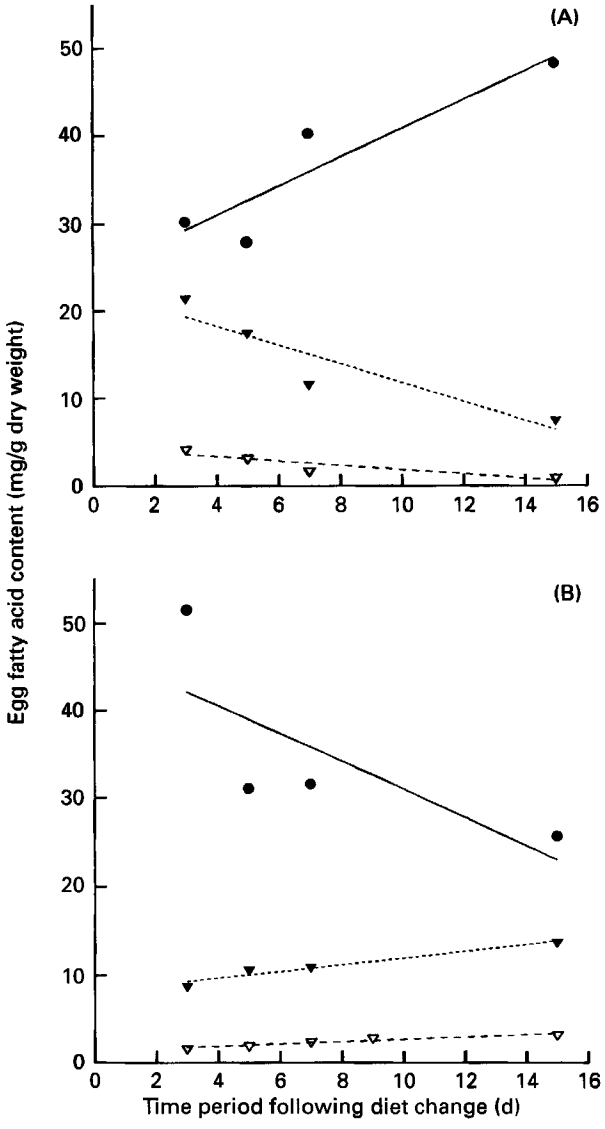

Fig. 4. Changes in essential fatty acids 18:2*n*-6 (●), 20:5*n*-3 (▽) and 22:6*n*-3 (▼) content in *Sparus aurata* eggs over the 15 d period from the broodstock dietary change of diet 1 (low in *n*-6:*n*-3 fatty acid ratio) to diet 3 (high in *n*-6:*n*-3 fatty acid ratio) (A), and vice versa (B). The lines drawn between the points were determined by first-order linear regression analysis of the data. Each data point represents the mean of egg samples from six spawning tanks.

The lipid composition of the broodstock diet affected egg quality in terms of percentage of viable buoyant eggs of the total number of eggs spawned (Fig. 6). When the level of *n*-3 HUFA in broodstock diet was high (7·5 mg/g dry weight of diet 1) or intermediate (4·2 mg/g dry weight of diet 2), about 50% of the total number of eggs spawned were viable. But when seabream previously given diet 1 were switched to diet 3 which had no *n*-3 HUFA, the percentage of viable buoyant eggs dropped within 10 d of feeding, and reached the same level as that of fish given diet 3 for 60 d. Fish fed on diet 3 which had no

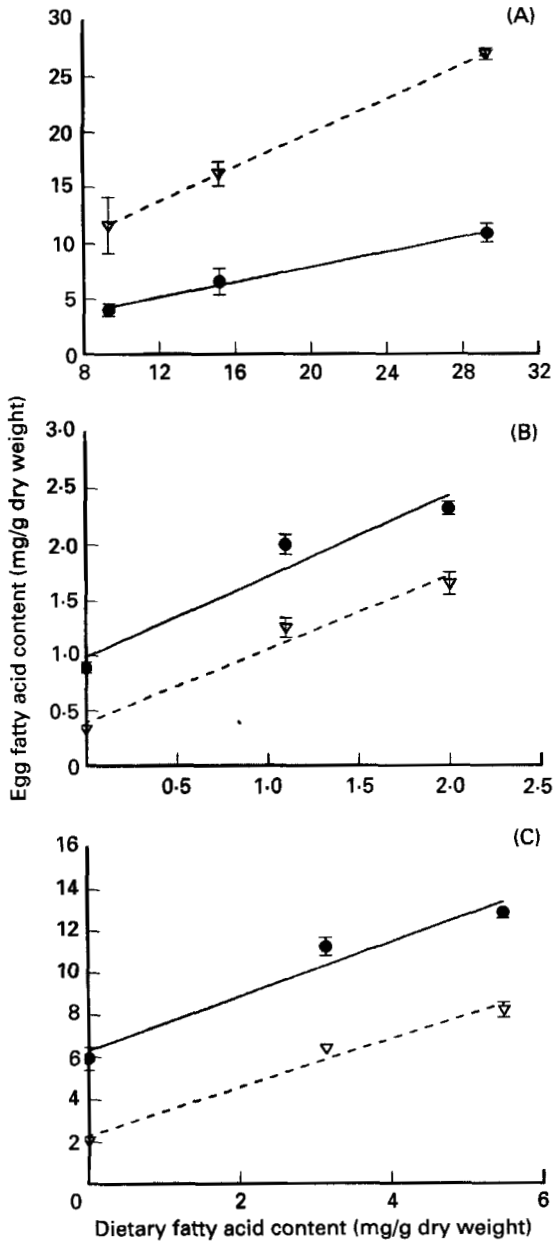

Fig. 5. The relationship between the levels of 18:2*n*-6 (A), 20:5*n*-3 (B), and 22:6*n*-3 (C), in the polar (●) and neutral (▽) fractions of egg lipid, after 60 d feeding, and their levels in *Sparus aurata* broodstock diet. The lines drawn between the points were determined by first-order linear regression analysis of the data (the equations of these regression lines are presented in Table 3.). Each data point represents the mean values with their standard errors of egg samples from six spawning tanks.

n-3 HUFA produced mostly dead eggs. However, when fish given this diet were switched to diet 1 which had a high level of *n*-3 HUFA the percentage of viable buoyant eggs increased dramatically after only 10 d feeding, and reached the same level as that of fish given diet 1 or 2 for 60 d.

Table 3. *Regression equations* for the relationship between content of the fatty acids 18:2n-6, 20:5n-3 and 22:6n-3 in the polar and neutral fractions of egg lipid and their content in* S. aurata *broodstock diet*

Fatty acid	Lipid fraction	*a*	*b*	*r*
18:2*n*-6	Neutral	0·77	4·48	1
	Polar	0·34	1·1	1
20:5*n*-3	Neutral	0·67	0·39	0·99
	Polar	0·73	0·93	0·97
22:6*n*-3	Neutral	1·14	2·29	0·99
	Polar	1·3	6·29	0·98

* Data shown are the parameters of first-order linear regression equations, *a* slope, *b* intercept and *r* correlation coefficient. All values are given in mg/g dry weight (*n* 6).

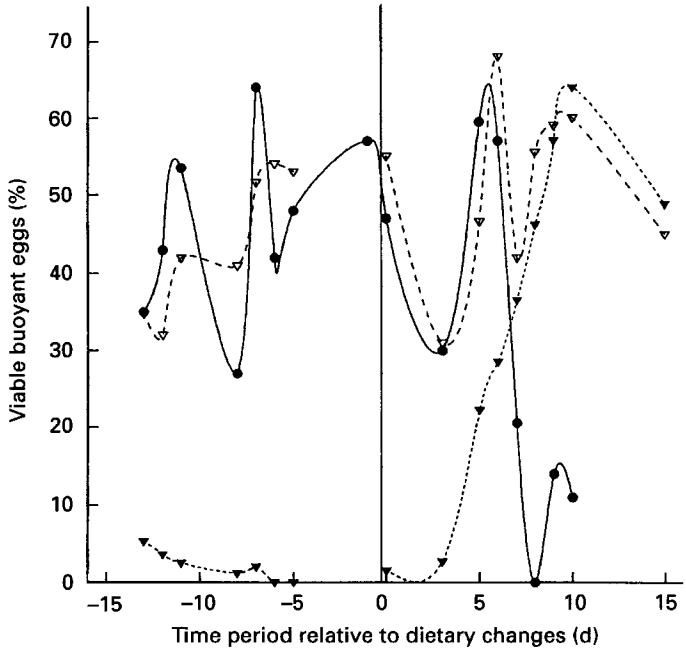

Fig. 6. Periodical changes in egg viability (percentage viable buoyant eggs of the total number of eggs spawned), over a 15 d period before and after the broodstock dietary change of diet 1 (●, high *n*-3 highly unsaturated fatty acids (HUFA)) to diet 3 (▼, no *n*-3 HUFA) and vice versa. Fish given diet 2 (▽, intermediate *n*-3 HUFA level) served as a control over the experimental period. Each data point represents the mean of egg samples from six spawning tanks.

Data presented in Figs 4 and 6 were combined and the relationship between the level of *n*-3 HUFA (20:5*n*-3 and 22:6*n*-3) in the egg from fish given all three diets and their viability in terms of percentage of viable buoyant eggs of the total number of eggs spawned is shown in Fig. 7. When the level of *n*-3 HUFA in the egg decreased below 17 mg/g dry weight, the percentage of viable buoyant eggs dropped sharply. On the other hand, higher *n*-3 HUFA level in the eggs did not result in a further significant improvement ($P > 0{\cdot}05$) in the percentage of viable eggs.

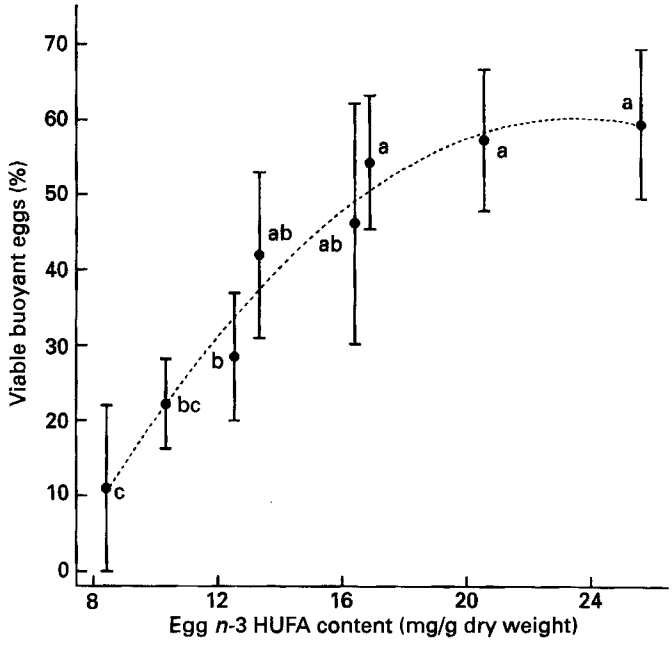

Fig. 7. The relationship between *n*-3 highly unsaturated fatty acids (HUFA; 20:5*n*-3 and 22:6*n*-3) in the eggs and their viability (percentage viable buoyant eggs of the total number of eggs spawned). The data is based on eggs collected over a 15 d period from the broodstock diet change, from diet 1 (high *n*-3 HUFA) to diet 3 (no *n*-3 HUFA) and vice versa. The best fit curvilinear line drawn between the points was determined by second-order linear regression analysis of the data (r 0·98). Each data point represents the mean values with their standard errors for egg samples from six spawning tanks. Points with different letters are significantly different from each other ($P < 0·05$).

DISCUSSION

All the diets used in the present study were similar in their energy content and were nutritionally adequate to sustain high fecundity in *S. aurata* broodstock. No effects of dietary composition on body weight, gonado-somatic index, fecundity, or tissue biochemical composition were observed during the experimental period.

The reproductive season of *S. aurata* can be divided into an ovarian growth phase occurring during the 2 months before spawning, and a spawning phase, lasting 3–4 months. The first phase is characterized by liver enlargement and extensive growth of the ovaries, without any significant changes in the biochemical composition and P:(S+M) ratio of the body tissues. During the ovarian growth phase, muscle tissue, which makes up the bulk of the body tissues, and the lipid-rich cartilage tissues (about 45%) do not assimilate lipids, as indicated by the conservative nature of fatty acid composition in these tissues compared with the dietary composition. This means that during ovarian growth of *S. aurata* no specific constituents are preferentially stored or utilized in the body tissues. The same results were observed in northern pike (*Esox lucius*) in which ovarian growth occurred without any depletion of body nutrients or somatic energy (Medford & Mackay, 1978; Diana & Mackay, 1979).

In the second phase, spawning is accompanied by fat depletion from muscle and digestive tract and its associated adipose tissues, resulting in a 13·2% decrease in body weight. The liver does not seem to play an important role in lipid storage or utilization during the spawning phase. In contrast to the fat depletion, no protein was depleted from the muscle, digestive tract, or gill cartilage tissues throughout the reproductive season, suggesting that

all amino acid requirements for metabolism and egg production are probably supplied from dietary sources. Similar inverse relationships between somatic and ovarian growth as well as fat and water content were noted during the reproductive cycle of coho salmon (*Oncorhynchus keta*) and Atlantic salmon (*Salmo salar*) (Hardy, 1984; Aksnes *et al.* 1986). The depletion of muscle lipid in these fish was due to low lipid biosynthetic ability coupled with high lipase activity in the liver (Ando *et al.* 1986; Hatano *et al.* 1989). The endogenous lipid which is mobilized from *S. aurata* muscle and digestive tract tissues during spawning is composed mostly of saturated and monounsaturated fatty acids, as indicated by the significant increase in the P:(S+M) ratio in these tissues. This observation suggests that the endogenous lipid utilized possibly serves mainly as an energy source in metabolism and to lesser extent for compensation of any broodstock dietary shortage in essential fatty acids. The low level (9·2 mg/g dry weight) of *n*-3 HUFA (20:5*n*-3 and 22:6*n*-3) found in the eggs of fish given no dietary *n*-3 HUFA (Fig. 5) was probably drawn from these endogenous sources, but it was far below the minimum requirement of 17 mg *n*-3 HUFA/g dry weight (Fig. 7).

The content of *n*-3 HUFA in *S. aurata* eggs can be increased only through dietary supplementation of these fatty acids, because marine fish lack the desaturation and elongation enzymes necessary to convert 18-carbon fatty acids to longer 20 and 22 carbon HUFA (Kanazawa *et al.* 1979; Sargent *et al.* 1989). Our results are in agreement with other studies on gilthead seabream (Mourente & Ordiozola, 1990*a*, *b*) and red seabream (Watanabe *et al.* 1984*d*) which showed that egg fatty acid composition reflects its composition in the broodstock diet. The observation that 18:2*n*-6 was consistently higher in the neutral than the polar fraction of the egg lipid, while *n*-3 HUFA (20:5*n*-3, 22:6*n*-3) were consistently higher in the polar fraction, can be explained by the fact that the *n*-3 HUFA supplemented in the diet had already undergone desaturation and elongation, and competed more efficiently with 18:2*n*-6 fatty acids for incorporation into the membrane phospholipids. Therefore, most of the dietary *n*-6 fatty acids are incorporated into the neutral fraction of the body tissues and egg lipids, and probably serve as an energy substrate, as evidenced by the low *n*-6:*n*-3 fatty acid ratio in the body tissues and eggs compared with the high dietary *n*-6:*n*-3 ratio.

The increase in 22:6*n*-3 in both polar and neutral fractions of the egg lipid in response to increasing broodstock dietary levels was 70% higher than the increase in 20:5*n*-3 and double that of the increase in 18:2*n*-6, suggesting preferential incorporation of 22:6*n*-3 into egg lipid. *S. aurata* egg phospholipids contain much higher levels of 22:6*n*-3 than 20:5*n*-3 or 18:2*n*-6, as reported also by Ando (1962) and Mourente & Ordiozola (1990*a*). This fatty acid may play an important role during the embryonic development of cellular and subcellular membranes of the brain and the central nervous system as well as photoreceptor cells of the retina, as was reported by many other studies in developing mice, piglets, rats, and chicks (Dianne & Innis, 1992; Wainwright *et al.* 1992).

The percentage viable buoyant eggs from the total number of eggs spawned, rate of hatching and survival up to 3 d from hatching were found to be sensitive indicators for evaluating egg quality in *S. aurata*, and other species which have pelagic eggs (Watanabe *et al.* 1984*a–d*; Sakai *et al.* 1985; Kjorsvik *et al.* 1990). All these factors demonstrated a high correlation with the broodstock dietary treatments. In the present study we report the results in terms of the percentage viable buoyant eggs, as this is a simple characteristic measurement which can be employed even at a very early stage of the embryonic development.

In the present study the dietary treatments differed from each other not only in their fatty acid composition but also in the level of their lipid classes and other fat-soluble components. However, since egg *n*-3 HUFA level reflects its broodstock dietary level, with

subsequent effect on egg viability, we conclude that *n*-3 HUFA in the eggs is probably responsible for egg quality. The results of our study suggest that *S. aurata* eggs should contain at least 17 mg *n*-3 HUFA/g dry weight in order to ensure their quality. In order to reach this level the *S. aurata* broodstock diet must contain at least 4·2 mg *n*-3 HUFA (mainly 22:6*n*-3)/g dry weight. The same requirement of 0·5% *n*-3 HUFA at 9% dietary lipid was observed during the grow-out phase of red seabream (Fujii & Yone, 1976).

In conclusion: *S. aurata* egg quality is very responsive to the nutritional composition of the broodstock diet. The expression of broodstock dietary changes in egg composition and egg quality occurred within 15 d. Mobilization of body stores of essential lipids during spawning can probably cover only minor deficiencies in *S. aurata* feed. A minimum deposition of 17 mg *n*-3 HUFA/g dry weight in the egg ensures its quality; such a level of deposition can be obtained if broodstock is given a dietary level of 4·2 mg *n*-3 HUFA/g dry weight.

REFERENCES

Aksnes, A., Gjerde, B. & Roald, S. O. (1986). Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture* **53**, 7–20.

Ando, K. (1962). Changes of the lipid during development of rainbow trout eggs. *Bulletin of Japanese Society for Scientific Fisheries* **28**, 73–76.

Ando, S., Yamazaki, F., Hatano, M. & Zama, K. (1986). Deterioration of chum salmon *Oncorhynchus keta* muscle during spawning migration 3. Changes in protein composition and protease activity of juvenile chum salmon muscle upon treatment with sex steroid. *Comparative Biochemistry and Physiology* **B 83**, 325–330.

Association of Official Analytical Chemists (1980). *Official Methods of Analysis* 13th ed. Arlington, VA: Association of Official Analytical Chemists.

Christie, W. W. (1982). *Lipid Analysis. Isolation, Separation, Identification and Structural Analysis of Lipids*, 2nd ed. Oxford: Pergamon Press.

Diana, J. S. & Mackay, W. C. (1979). Timing and magnitude of energy deposition and loss in body, liver, and gonads of northern pike *Esox lucius*. *Journal of the Fisheries Research Board of Canada* **36**, 481–487.

Dianne, L. A. & Innis, S. M. (1992). Docosahexaenoic acid in developing brain and retina of piglets fed high or low α-linolenate formula with and without fish oil. *Lipids* **27**, 89–93.

Folch, J., Lees, M. & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Fryer, H. C. (1966). *Concepts and Methods of Experimental Statistics*. Boston, MA: Allyn & Baccon.

Fujii, M. & Yone, Y. (1976). Studies on nutrition of red seabream. XIII. Effect of dietary linolenic acid and *n*-3 polyunsaturated fatty acids on growth and feed efficiency. *Nippon Suisan Gakkaishi* **42**, 583–588.

Gothilf, Y. (1990). Pharmacokinetics, metabolism and bioactivity of gonadotropin releasing hormone (GnRH) and its analogs in the gilthead seabream *Sparus aurata*. M.Sc. Thesis, Hebrew University of Jerusalem.

Hardy, R. W. (1984). Salmonid broodstock nutrition. *Aquaculture* **43**, 98–108.

Hatano, M., Mizogami, M. & Sugawara, A. (1989). Lipid metabolism. *Nippon Suisan Gakkaishi* **55**, 1623–1627.

Kanazawa, A., Teshima, S. I. & Ono, K. (1979). Relationship between essential fatty acid requirements of aquatic animals and the capacity of bioconversion of linolenic acid to highly unsaturated fatty acids. *Comparative Biochemistry and Physiology* **B 63**, 295–298.

Kjorsvik, E., Mangor, A. J. & Holmefjord, I. (1990). Egg quality in fishes. *Advances in Marine Biology* **26**, 71–113.

Lal, B. & Singh, T. P. (1987). Changes in tissue lipid levels in the freshwater catfish *Calarias batrachus* associated with the reproductive cycle. *Fish Physiology and Biochemistry* **3**, 191–201.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Medford, B. A. & Mackay, W. C. (1978). Protein and lipid content of gonads, liver, and muscle of northern pike (*Esox lucius*) in relation to gonad growth. *Journal of the Fisheries Research Board of Canada* **35**, 213–219.

Morrison, W. R. & Smith, L. M. (1964). Preparation of fatty acid esters and dimethylacetals from lipids with boron fluoride–methanol. *Journal of Lipid Research* **5**, 600–608.

Mourente, G. & Odriozola, J. M. (1990*a*). Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead seabream *Sparus aurata* L. *Fish Physiology and Biochemistry* **8**, 93–101.

Mourente, G. & Odriozola, J. M. (1990*b*). Effect of broodstock diets on total lipids and fatty acid composition of larvae of gilthead seabream *Sparus aurata* L. *Fish Physiology and Biochemistry* **8**, 103–110.

Nassour, I. & Leger, C. L. (1989). Deposition and mobilization of body fat during sexual maturation in female trout *Salmo gairdneri* R. *Aquatic Living Resources* **2**, 153–159.

Sargent, J. R., Henderson, R. J. & Tocher, D. R. (1989). The lipids. In *Fish Nutrition*, pp. 153–218 [J. E. Halver, editor]. New York, London: Academic Press.

Sakai, K., Nomura, M. & Takashima, F. (1985). Characteristics of naturally spawned eggs of red seabream. *Bulletin of Japanese Society for Scientific Fisheries* **51**, 1395–1399.

Shimma, Y., Suzuki, R., Yamaguchi, M. & Akiyama, T. (1977). The lipids of adult carps raised on fish meal and SCP feeds and hatchabilities of their eggs. *Bulletin of Freshwater Fisheries Research Laboratory* **27**, 35–48.

Sokal, R. R. & Rholf, F. J. (1981). *Biometry*. San Francisco: W. H. Freeman and Company.

Wainwright, P. E., Huang, Y. S., Fleming, B. B., Dalby, D., Mills, D. E., Redden, P. & McCutchen, D. (1992). The effects of dietary *n*-3/*n*-6 ratio on brain development in the mouse: a dose response study with long-chain *n*-3 fatty acids. *Lipids* **27**, 98–103.

Washburn, B. S., Frye, D. J., Hung, S. I. & Conte, F. S. (1990). Dietary effects on tissue composition, oogenesis and the reproductive performance of female rainbow trout *Oncorhynchus mykiss*. *Aquaculture* **90**, 179–195.

Watanabe, T., Arakawa, T., Kitajima, C. & Fujita, S. (1984*a*). Effect of nutritional quality of broodstock diet on reproduction of red seabream. *Bulletin of Japanese Society for Scientific Fisheries* **50**, 495–501.

Watanabe, T., Itoh, A., Murakami, A., Tsukashima, Y., Kitajima, C. & Fujita, S. (1984*b*). Effect of dietary protein level on reproduction of red seabream. *Bulletin of Japanese Society for Scientific Fisheries* **50**, 1015–1022.

Watanabe, T., Itoh, A., Murakami, A., Tsukashima, Y., Kitajima, C. & Fujita, S. (1984*c*). Effect of nutritional quality of diets given to broodstock on the verge of spawning on reproduction of red seabream. *Bulletin of Japanese Society for Scientific Fisheries* **50**, 1023–1028.

Watanabe, T., Ohhashi, S., Itoh, A., Kitajima, C. & Fujita, S. (1984*d*). Effect of nutritional composition of diets on chemical components of red seabream broodstock and egg produced. *Bulletin of Japanese Society for Scientific Fisheries* **50**, 503–515.

Watanabe, T., Takeuchi, T., Saito, M. & Nishimura, K. (1984*e*). Effect of low protein–high calorie or essential fatty acid deficient diet on reproduction of rainbow trout. *Bulletin of Japanese Society for Scientific Fisheries* **50**, 1207–1215.

Watanabe, T., Itoh, A., Satoh, S., Kitajima, C. & Fujita, S. (1985*a*). Effect of dietary protein levels and feeding period before spawning on chemical compositions of eggs produced by red seabream. *Bulletin of Japanese Society for Scientific Fisheries* **51**, 1501–1509.

Watanabe, T., Koizumi, T., Suzuki, H., Satoh, S., Takeuchi, T., Yoshida, N., Kitada, T. & Tsukashima, Y. (1985*b*). Improvement of quality of red seabream eggs by feeding broodstock on a diet containing cuttlefish meal or a raw krill shortly before spawning. *Bulletin of Japanese Society for Scientific Fisheries* **51**, 1511–1521.

Printed in Great Britain