Relationship between egg quality and fatty acid content of various turbot broodstocks (*Scophthalmus maximus* L.)

J. B. Peleteiro, P. Lavens, G. Rodríguez-Ojea, and J. Iglesias

The paper presents the data relating to the spawning quality of 20 turbot females (*Scophthalmus maximus* L.) subjected to natural and controlled photoperiods and fed with two different types of food. A comparison is made between the data usually utilized in hatcheries for defining the spawning quality (number of viable eggs, fertilization and hatching rates, number of larvae produced) and the highly unsaturated fatty acid (HUFA) content of fertilized eggs at the two-cell stage. The results indicate that there is no correlation between the HUFA content of the eggs and any of the egg quality criteria examined. Nevertheless, significant differences ($p < 0.05$) are found between the females fed with fresh trash fish (higher 18:3n-3, 20:4n-6, total lipid content and the ratio DHA/EPA) and those fed with commercial pellets.

J. B. Peleteiro, G. Rodríguez-Ojea, and J. Iglesias: Instituto Español de Oceanografía, Apdo. 1552, 36280-Vigo, Spain [tel: (+34) 86 492111, fax: (+34) 86 492351]. P. Lavens: Laboratory of Aquaculture and Artemia Reference Center, University of Gent, Rozier 44, B-9000 Gent, Belgium [tel: (+32) 91 643754, fax: (+32) 91 644193].

Introduction

In the last decade it has been demonstrated extensively that highly unsaturated fatty acids are an essential nutrient for early larval development (Fraser *et al.*, 1987; Watanabe, 1993). Maternal nutrition affects the egg nutrient composition (Lavens and Sorgeloos, 1991) and consequently egg and larval quality, since throughout this period they rely on the endogenous energy reserves (Watanabe, 1993).

The sampling strategy applied in this work enabled us to check whether certain broodstock conditions affect the nutrient content of the eggs. In particular, factors which are generally accepted to have an effect on egg quality were considered, e.g. photoperiod control vs natural breeding conditions, part of the reproductive period, ripe vs overripe eggs, etc. Egg quality was evaluated in accordance with the data usually utilized in hatcheries for this purpose. We therefore attempt in this work to find a correlation between any of these parameters and the egg nutrient composition.

Materials and methods

Two turbot broodstocks from the Spanish Oceanographic Institute in Vigo were kept under different feeding and light conditions. One group was fed twice a week with semi-dry pellets made with a mixture of fish oil, fish meal, water, and a supplement of vitamins A, C, and E, giving a final composition of 29.4% protein, 10.2% lipid, 42.9% humidity, and 6.9% ash content.

This broodstock was subjected to photoperiod control. From June 1990 to January 1991 the light was maintained constant for $8\,h\,day^{-1}$. In January 1991 the photoperiod was increased suddenly from 8 to $16\,h\,day^{-1}$, and maintained at this level until spawning ended. The first egg batches were obtained after about 2 months.

The second broodstock was also fed twice a week with fresh trash fish, with a basic composition of 25% blue fish and 75% white fish. This group was subjected to natural photoperiod.

The two diets analysed in this experiment are those

usually utilized in our hatchery and in the commercial turbot farms in northern Spain.

Eggs and milt were obtained by hand-stripping, and the dry fertilization method was used (Forés *et al.*, 1990). Sea water was added after 15 min, and the number of buoyant and non-buoyant eggs and the percentage fertilization were calculated after 3–4 h, when eggs are in the two-cell stage. At this moment, a small sample (200 mg dry wt.) of eggs was taken from the floating layer of eggs for biochemical analysis.

The rest of the buoyant eggs were then incubated in a 150 litre cylindrical-conical incubator with open-water circulation and mild aeration. The incubation temperature was kept between 13 and 15°C. At the end of incubation (4–5 days), the percentage hatch and number of larvae were calculated.

Eggs were classified in two groups of different quality (good and medium) according to the morphological criteria cited by Kjørsvik *et al.* (1990), including the symmetry of the early blastomeres, egg tansparency, and size of perivitelline space.

Buoyancy of the eggs after artificial fertilization was the only criterion used for classifying the eggs as ripe (buoyant) and overripe (non-buoyant).

Egg samples were stored at −80°C until freeze-drying. Fatty acids were analysed by gas chromatography as described by Léger *et al.* (1991).

Results

The spawning characteristics and fatty acid composition of all the analysed egg samples are presented in Table 1. The results show a high variation for most of the fatty acid classes among the egg samples. Calculation of the variation coefficients gives values of 20% for EPA, and more than 35% for DHA and the sum of n-3 HUFA. Even the total lipid content varies by more than 20%, which seems to be very high, since this is a major energy source as well as an essential component for the development of membrane structures during early larval development. The fatty acid concentrations, too, vary tremendously.

This table shows that there is no relationship between any of the criteria utilized to describe the spawning quality and the fatty acid content. For example, some of the lowest values in fertilization, hatching, and larvae produced (females 108, 52 and 58) correspond to high levels in DHA, Σ HUFA, and percentage total lipids, while high values in those three characteristics (females, 17, 55, and 2) correspond to low DHA and Σ HUFA content. Even two different spawns of female 5, with a very different viable eggs rate, have similar fatty acid values.

When trying to see if certain broodstock conditions interfere with the nutrient content of the eggs, Table 2 shows that only in a very few cases are there differences in fatty acid composition between any of the groups selected. When comparing these parameters it is clear that no correlation can be drawn with the fatty acid composition or total lipid content of the eggs. However, when broodstock feeding conditions are investigated (see also Fig. 1) it can be noted that significant differences (ANOVA p < 0.05) are detected between the samples obtained from females fed with pellets and those from females fed with fresh fish: the former have a lower 18:3n-3, 20:4n-6, percentage total lipids and DHA/EPA ratio than the latter.

Because of these differences in fatty acid content due to the diet, we finally tried to see whether the broodstock feeding conditions also affect spawning characteristics such as buoyancy, fertilization, and hatching percentages. No significant differences were found (p < 0.05 ANOVA test).

Discussion and conclusions

It is striking that a high variability in fatty acid composition of the turbot eggs is detected. However, the average values of HUFA content, more specifically EPA and DHA, and the ratios DHA/EPA and n-3/n-6 are comparable to those reported by Witt *et al.* (1984) and even substantially higher than those given by Planas *et al.* (1989). Lavens and Sorgeloos (1991) compared several broodstocks, also finding higher levels for HUFA and DHA in French turbot eggs compared to our samples, which is in agreement with the data of Rubio Rincón (1986). Apparently, there is no clear reason for these differences, especially since the same broodstock conditions result in a high variability, e.g., DHA ranges maximally ±12 mg.

One would expect that the differences noted would also interfere with the egg quality, since Planas *et al.* (1989) suggest that (n-3) HUFA play an important role just after hatching and that some fatty acids are the main energy source during embryogenesis. The strategy of the sampling campaign enabled us to check whether certain broodstock conditions interfere with the nutrient content of the eggs and, finally, egg and larval quality. In particular, factors which are generally accepted to have a possibly negative impact on egg quality (Kjørsvik *et al.*, 1990) were considered. It can be concluded from these data that no correlation can be drawn between the fatty acid composition of the eggs, on the one hand, and the production conditions examined or the egg and larval viability, on the other.

However, there is one exception: significant (ANOVA p < 0.05) differences could be detected between eggs obtained from stocks fed on artificial diet and

Table 1. Spawning characteristics and fatty acid composition of different egg batches (DHA = 22:6n-3; EPA = 20:5n-3; Σ HUFA = n-3 highly unsaturated fatty acids >20:3n-3). The first 13 batches are from the broodstock fed with pellets, and the last 9 from the broodstock fed with trash fish.

| Batch | Female | Buoyant eggs (×10) | Sinking eggs (×10) | % Fert. | % Hatch. | Larvae hatched | mg/gDW | | | | | | Total lipids (% D.W.) | DHA/EPA | n-3/n-6 |
							18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3	HUFA			
RB-1	5	90	0	92	2.1	1875	4.1	1.7	0.9	7.2	16.5	25.8	10.37	2.29	3.93
–	5	10	238	0			4.2	1.5	0.9	7.0	17.2	26.3	15.09	2.46	3.92
RB-3	10	247	34	55	0	0	3.0	2.3	0.6	5.4	13.8	21.5	15.46	2.55	3.45
RB-4	18	268	0		10.1	30000	3.5	1.8	0.7	5.4	14.7	22.5	17.79	2.72	3.63
RB-10	67	162	0	60	13.3	19400	3.8	1.5	0.7	6.9	15.7	24.6	12.21	2.27	3.84
RB-12	17	112	13	92	52.4	59000	3.3	2.3	0.4	5.9	12.3	19.9	13.33	2.08	3.04
RB-16	39	112	99	65	29	26200	3.1	1.8	0.6	5.2	12.9	19.7	12.68	2.48	3.36
RB-19	55	247	49	97	70.7	164400	3.1	2.2	0.6	6.3	12.3	20.1	12.74	1.95	3.38
RB-24	99	112	144	97	28.9	32500	3.3	1.3	0.7	6.9	15.1	23.8	12.37	2.19	4.48
RB-30	53	90	18	87	60.6	29300	3.9	1.9	1.0	8.4	20.4	31.7	12.57	2.43	4.48
RB-36	9	157	49	91			4.0	2.3	0.7	7.5	17.3	27.5	18.93	2.31	3.97
–	41	40	81				3.7	2.5	0.9	6.4	18.1	27.4	15.14	2.83	3.4
RB-46	108	90	63	55	1	1000	4.7	3.0	0.8	7.5	16.8	26.6	21.48	2.24	2.82
–	S.N.	–	–	21			5.4	2.2	1.1	8.3	16.6	27.6	25.09	2.00	3.14
RB-53	2	112	22	85	44.9	46500	3.4	3.2	2.4	5.2	13.3	20.5	37.28	2.56	2.14
RB-62	54	135	85	94	23.8	33300	1.8	1.7	0.9	5.4	16.0	24.0	14.73	2.96	4.36
RB-68	52-H	76	16	67	0	0	3.2	3.0	1.0	6.4	20.0	28.7	17.97	3.12	3.48
–	58	67	25	3			3.8	3.4	1.2	6.5	24.5	33.7	20.97	3.77	3.86
–	7	151	87	28			2.9	2.7	0.9	5.9	17.9	25.8	17.3	3.03	3.48
RB-70	104	45	135	65	40	20000	2.2	3.6	2.3	6.3	18.3	27.6	15.25	2.90	2.74
RB-72	16	117	211	72	6.6	6450	2.3	2.3	0.9	5.3	13.8	21.1	18.59	2.60	3.3
RB-73	77	45	11	87	38.4	14300	1.5	2.3	2.0	5.1	13.6	21.6	13.6	2.67	2.66

Table 2. Fatty acid composition of turbot eggs according to certain broodstock conditions. The results are presented as mean values ±s.d. Significant differences (p < 0.05) were only found where indicated in bold type (groups with less than three samples were not compared).

|		mg/gDW						Total lipids (% DW)	DHA/EPA	n-3/n-6	Samples
		18:2n-6	18:3n-3	20:4n-6	20:5n-3	22:6n-3	HUFAS				
Feed	Pellets	3.7 ± 0.5	**2.0 ± 0.5**	**0.7 ± 0.2**	6.6 ± 1.0	15.6 ± 2.4	24.4 ± 3.6	**14.63 ± 3.15**	**2.37 ± 0.24**	3.67 ± 0.50	13
	Fresh fish	2.9 ± 1.2	**2.7 ± 0.6**	**1.4 ± 0.6**	6.0 ± 1.0	17.1 ± 3.6	25.6 ± 4.3	**20.09 ± 7.33**	**2.85 ± 0.48**	3.24 ± 0.67	9
Breeding season	Beginning	3.6 ± 0.9	2.2 ± 0.7	1.0 ± 0.5	6.4 ± 1.0	16.8 ± 3.3	25.5 ± 3.9	18.70 ± 7.77	2.67 ± 0.51	3.58 ± 0.60	10
	Middle	3.0 ± 0.8	2.3 ± 0.6	1.0 ± 0.6	6.3 ± 1.0	15.6 ± 2.9	24.2 ± 4.0	14.77 ± 2.47	2.50 ± 0.35	3.48 ± 0.61	11
	End	4.7	3.0	0.8	7.5	16.8	26.6	21.48	2.24	2.82	1
Female spawning cycle	Early	3.3 ± 0.9	2.2 ± 0.6	0.9 ± 0.6	6.3 ± 0.9	**15.0 ± 2.1**	23.5 ± 3.0	14.78 ± 3.18	**2.38 ± 0.26**	3.45 ± 0.55	13
	Middle	3.2 ± 0.7	2.5 ± 0.7	1.2 ± 0.6	6.1 ± 1.1	**18.1 ± 3.8**	26.7 ± 4.9	19.80 ± 8.15	**2.94 ± 0.44**	3.63 ± 0.77	7
	End	3.7	2.5	0.9	6.4	18.1	27.4	15.14	2.83	3.40	1
Egg quality	Good	3.2 ± 0.9	**2.0 ± 0.5**	1.0 ± 0.6	6.4 ± 1.1	15.0 ± 2.5	23.6 ± 3.8	17.6 ± 9.7	**2.38 ± 0.28**	3.60 ± 0.84	11
	Medium	3.3 ± 0.8	**2.6 ± 0.7**	1.0 ± 0.5	6.2 ± 0.7	17.5 ± 3.2	26.1 ± 3.8	17.5 ± 2.4	**2.82 ± 0.43**	3.42 ± 0.36	10
Egg stage	Ripe	3.3 ± 0.9	2.3 ± 0.6	1.0 ± 0.6	6.4 ± 1.0	16.1 ± 3.1	24.7 ± 4.0	17.04 ± 6.07	2.56 ± 0.44	3.48 ± 0.62	20
	Overripe	4.0 ± 0.4	2.0 ± 0.7	0.9 ± 0.0	6.7 ± 0.4	17.7 ± 0.6	26.9 ± 0.8	15.12 ± 0.04	2.65 ± 0.26	3.66 ± 0.37	2
All samples		3.4 ± 0.9	2.3 ± 0.6	1.0 ± 0.5	6.4 ± 1.0	16.2 ± 2.9	24.9 ± 3.8	16.86 ± 5.67	2.56 ± 0.43	3.49 ± 0.58	22
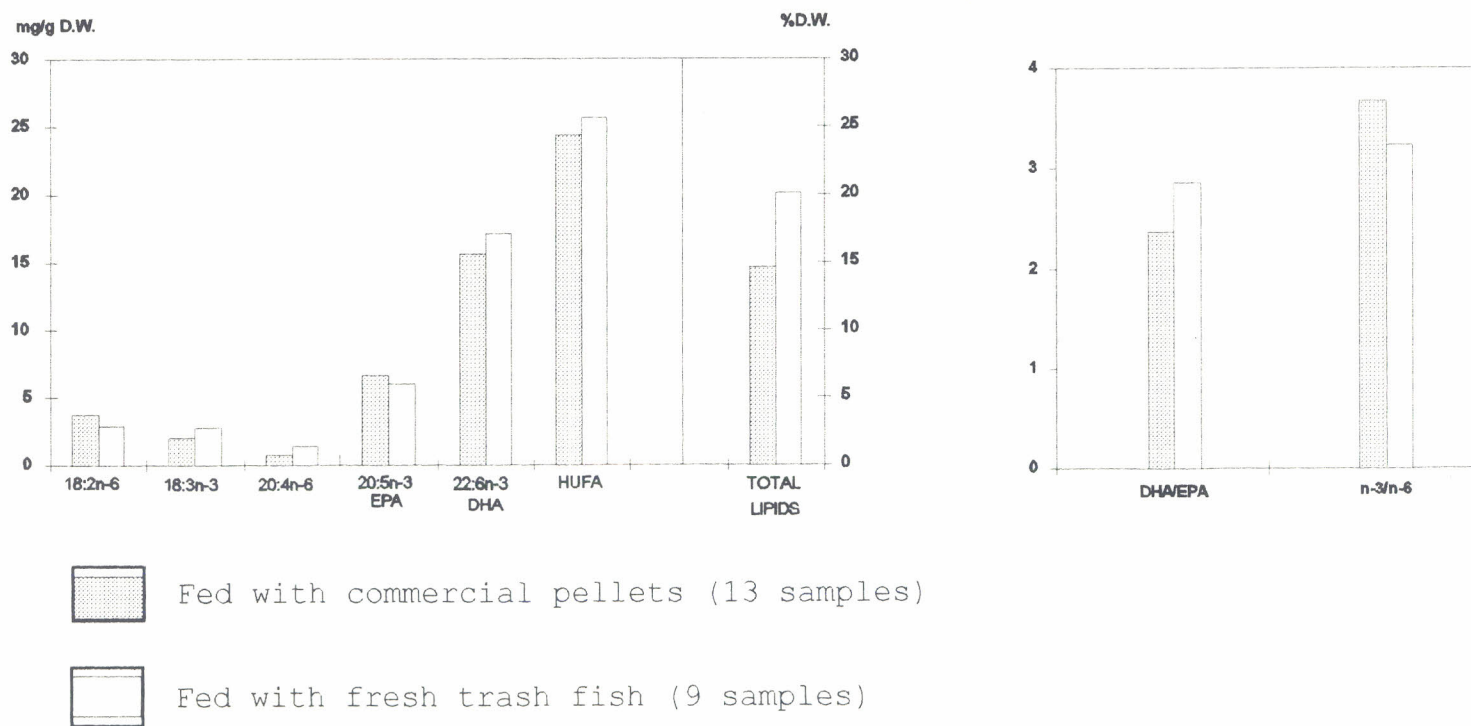
Figure 1. Fatty acid composition of turbot eggs obtained from females submitted to different feeding conditions. There are significant differences (p < 0.05) in 18:3n-3, 20:4n-6, percentage total lipids and DHA/EPA ratio.

on fresh trash fish. The first contain lower levels of 18:3n-3, 20:4n-6 DHA, and total lipids. Broodstock nutrition therefore affects the biochemical composition of the offspring, as was also demonstrated by Watanabe (1991) for red sea bream. However, using fatty acid composition as a criterion for egg or larval quality did not show any correlation again.

This implies that all observed minimum levels of the essential fatty acids EPA and DHA seem to be sufficiently high to meet the requirements for normal embryonic development. Labarta *et al.* (1989) report that optimal egg quality in turbot is achieved when 16:0, 18:1n-9, and 22:6n-3 levels are respectively 1.8, 1.4 and 1.7% of the egg dry weight. Unfortunately, we do not have sufficient data available on start-feeding success for any conclusions on overall larval quality to be drawn. Moreover, the data of the different broodstock feeding should be interpreted with care, since also another culture parameter is changing: natural or photoperiodical control of the reproduction cycle.

Although some authors (Planas *et al.*, 1991) report that a shift of the spawning period through photoperiodic control results in a decrease of n-3 PUFA and n-9 fatty acids, Forés *et al.* (1990), working with the same broodstock as in our experiment, found that a dramatic change in the photoperiod produced eggs of good quality with a similar percentage of fertilization and hatch to those spawned by natural photoperiod. Because of this, we consider that it is the broodstock diet composition and not the photoperiodic control which really affects the egg lipid content.

References

Forés, R., Iglesias, J., Olmedo, M., Sánchez, F. J., and Peleteiro, J. B. 1990. Induction of spawning in turbot (*Scophthalmus maximus* L.) by a sudden change in the photoperiod. Aquacult. Engineering, 9: 357–366.

Fraser, A. J., Sargent, J. R., Gamble, J. C., and Maclachlan, P. 1987. Lipid class and fatty acid composition as indicators of the nutritional condition of larval Atlantic herring. Am. Fish. Soc. Symp. 2: 129–143.

Kjørsvik, E., Mangor-Jensen, A., and Homefjord, I. 1990. Egg quality in fishes. *In* Adv. Mar. Biol., 26: 71–113.

Labarta, U., Planas, M., Ferreiro, M. J., Fernández-Reiriz, M. J., and Garrido, J. L. 1989. Biochemical factors affecting egg quality in turbot: a general approach. ICES Working Group on Mass Rearing of Juvenile Marine Fish. Montpellier, France, June 1989.

Lavens, P., and Sorgeloos, P. 1991. Variation in egg and larval quality in various fish and crustacean species. *In* Larvi '91 – Fish and Crustacea Larviculture Symposium. Eur. Aquacult. Soc., Spec. Publ. No. 15: 221–222.

Léger, P., Bengtson, D. A., and Sorgeloos, P. 1989. Analytical variation in the determination of the fatty acid composition of standard preparations of brine shrimp *Artemia*: an interlaboratory exercise, pp. 413–423. *In* Aquatic toxicology and hazard assessment: 12th volume, ASTM STP 1027, U. M. Cowgill and L. R. Williams (Eds.) American Society for Testing and Materials, Philadelphia, USA.

Planas, M., Ferreiro, M. J., Fernández-Reiriz, M. J., and Labarta, U. 1989. Evolución de la composición bioquímica y actividades enzimáticas en huevos de rodaballo (*Scophthalmus maximus* L.) durante la embriogénesis. *In* Acuacultura Intermareal, pp. 215–227. Ed. by M. Yúfera. Inst. Cien. Andalucía, Cádiz.

Planas, M., Garrido, J. L., Labarta, U., Fernández-Reiriz, M. J., Rodríguez, J. L., and Otero, J. J. 1991. Biochemical characteristics of the eggs of farmed turbot (*Scophthalmus maximus* L.). *In* Larvi '91 – Fish and Crustacen Larviculture Symposium. Eur. Aquacult. Soc., Spec. Publ. No. 15: 207–209.

Rubio Rincón, E. A. 1986. Evolution de la composition en acides gras de l'ovocyte à la larve du turbot (*Psetta maxima* L.) en fonction du Régime alimentaire des reproducteurs et des larves, ainsi que de la température d'incubation. PhD thesis, Université de Bretagne Occidentale. 183 pp.

Watanabe, T. 1991. Axtaxanthin: an effective dietary component for red seabream broodstock. *In* Fish nutrition in practice, book of abstracts. IV International Symposium on Fish Nutrition and Feeding. Biarritz, France.

Watanabe, T. 1993. Importance of Docosahexaenoic acid in marine larval fish. *In* Larvi '91 – Fish and Crustacean Larviculture Symposium. Eur. Aquacult. Soc., Spec. Publ. No. 15: 19.

Witt, U., Quantz, G., Kuhlmann, D., and Kattner, G. 1984. Survival and growth of turbot larvae *Scophthalmus maximus* L. reared on different food organisms with special regard to long-chain polyunsaturated fatty acids. Aquacult. Engineering, 3: 177–190.