Seafish Industry Authority

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Final Project Report

Project details

Seafish Project Code	63407		
Project Title	Fatty acid and carotenoid content of Atlantic cod eggs from Scottish hatcheries: effects on egg and larval quality.		
Name and address of contractor	Institute of Aquaculture, University of Stirling, Stirling FK9 4LA		
Total cost to Seafish	£5000	**Total project cost**	£10000
Project start date	01/10/2007	**Project end date**	31/7/2008

Executive Summary

Maximum of 2 sides A4. Should provide a layman's summary of the conduct and result of the project.

Production of good quality juveniles from cod hatcheries relies heavily on the quality of eggs produced. However, egg quality can be highly variable and identifying, at an early stage, which eggs are likely to generate good hatch rates and larval survival can save time and money that could be wasted incubating poor quality eggs. Evidence suggests that the egg fatty acid composition, especially arachidonic acid (ARA) and docosahexaenoic acid (DHA) concentrations, as well as the ARA/eicosapentaenoic acid (EPA) ratio are vital for good egg quality. Similarly the egg carotenoid, principally astaxanthin, (Ax) content is also correlated with improved egg and larval quality in other fish species. The key project objectives were as follows;

Objective 1; Determination of egg lipid content and fatty acid composition especially ARA, DHA and the EPA/ARA ratio, in relation to egg quality in UK cod hatcheries.

Objective 2; Determination of carotenoid content in cod eggs in relation to egg quality in UK cod hatcheries.

Objective 3; To investigate the use of maternal blood lipid compositions as predictors of egg fatty acid compositions in Atlantic cod.

Twelve egg batches were received and analysed from Machrihanish Marine Farm Ltd (MMF) that were collected between 4/10/07 and 24/10/07. Ninety two egg batches were received from Viking Fish Farms (VFF) that were collected between 4/10/07 and 29/5/08. Originally it was planned to collect 50 egg batches from each of the two hatcheries but operational problems at MMF prevented any egg collection after October 2007. However, the total number of egg batches collected from the two sites was 104 so the total egg batches analysed exceeded the planned target of 100 batches.

The average number of eggs per batch produced and the average weights per batch of sinking and floating eggs were all significantly higher in the MMF groups. However, this could be related to differences in fish size and/or biased by the small number of egg batches analysed from MMF, but may also be related to broodstock feed quality with respect to lipid quality and carotenoid content. The % of floating eggs was higher in the VFF samples compared to the MMF samples (72 vs 54% respectively) while average fertilisation rate was not different between the two hatcheries. Differences in % floating eggs may be related to the tank hydrodynamics rather than batch quality.

The carotenoid pigment, astaxanthin (Ax), was significantly elevated in the MMF eggs compared to those from VFF with the former being 3-fold higher than the latter. Literature evidence suggests that increased egg carotenoid concentrations are linked to improved egg and larval quality in Atlantic cod. This effect could be linked to the well documented antioxidant activity provided by carotenoid pigments that has been described in both fish and mammals.

The MMF eggs were significantly higher in ARA, lower in EPA and the EPA/ARA ratio was subsequently lower compared to the VFF eggs. Literature evidence suggests that ARA in egg lipids is higher, compared to maternal lipids, in many fish species and that increased egg ARA and reduced EPA/ARA ratio provides improvements in egg and larval quality. This might explain the improvements in egg quality observed in the present study but this could not be confirmed by analysis of more samples from MMF. Tissue fatty acids are closely linked to dietary fatty acids and composition of the MMF and VFF moist diets showed the relationship of dietary fatty acid compositions on egg compositions at the two hatcheries**.**

Egg samples were obtained from VFF between October 2007 and May 2008 and allowed a comparison of egg quality, carotenoid and lipid concentrations between stocks spawning in autumn, spring and summer. There was an increased number of sinking eggs recorded in the autumn spawners compared to spring and summer groups. In contrast, the % of floating eggs was highest in the spring spawners with lowest levels in summer spawners. The highest concentrations of ARA were seen in the spring spawners and the lowest in autumn spawners while egg Ax concentrations were highest in the autumn spawners, although there was considerable variation between batches across all seasons.

A comparison of egg quality parameters and egg Ax and fatty acid concentrations was made across the eight broodstock tanks used at VFF. Significant variation was seen between egg quality parameters, Ax and fatty acid concentrations between the different tanks. Graphs of fertilisation rate plotted against egg ARA content and the egg EPA/ARA ratio showed that fertilisation rate was correlated with both egg ARA and EPA/ARA ratio (R^2 = 0.46 and 0.44, respectively). No correlations were found between Ax and fertilisation rate, total egg production, floating egg production or ARA and total egg production or total floating egg production.

While improvements in broodstock performance can be affected by individual nutrients it is also likely that broodstock source and genetics, season of sampling and use of photoperiod as well as diet and other environmental conditions also influence overall performance and these are all worthy of further study to elucidate their individual and collective roles in egg quality.

The final stage of the project investigated whether maternal blood lipids could act as predictors of egg fatty acid concentrations and ratios. Samples of blood and eggs, obtained by hand stripping, were collected from 32 broodstock at MMF in February and March 2008. The blood was separated into red blood cell (RBC) and plasma fractions by and the eggs, RBC and plasma fatty acid compositions were determined.

Plots of egg and blood highly unsaturated fatty acids (HUFA) were analysed using linear regression to establish whether egg HUFA could be correlated with blood HUFA. These data suggest that the EPA/ARA ratio in RBC could be used as a fairly accurate predictor of the EPA/ARA ratio in the eggs. In general, the blood ARA concentrations were better indicators of egg ARA than similar associations between EPA and DHA.

Scientific Report

Project title	Fatty acid and carotenoid content of Atlantic cod eggs from Scottish hatcheries: effects on egg and larval quality.

Maximum of 20 sides of A4, including bibliography. Additional information may be included as separate appendices, which may not be published.

The key project objectives were as follows;

Objective 1; Determination of egg lipid content and fatty acid composition especially ARA, DHA and the EPA/ARA ratio, in relation to egg quality in UK cod hatcheries.

Objective 2; Determination of carotenoid content in cod eggs in relation to egg quality in UK cod hatcheries.

Objective 3; To investigate the use of maternal blood lipid compositions as predictors of egg fatty acid compositions in Atlantic cod.

Results

Twelve egg batches were received and analysed from Machrihanish Marine Farms Ltd (MMF) that were collected between 4/10/07 and 24/10/07. Ninety two egg batches were received from Viking Fish Farms (VFF) that were collected between 4/10/07 and 29/5/08. In the original plan 50 egg batches were supposed to be collected from each of the two sites but operational problems at MMF prevented egg collection after October 2007. However, the total number of egg batches collected from the two sites was 104 so the total egg batches analysed did exceed the planned target.

The main essential fatty acids arachidonic acid (20:4n-6, ARA), eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) are shown in **Table 1** along with the ratios of DHA/EPA and EPA/ARA as well as measures of egg quality. The values were compared statistically using the Kruskal-Wallis non-parametric test.

Table 1. A comparison of egg quality parameters and fatty acid concentrations in egg batches collected from MMF and VFF in 2007 and 2008.

	Viking farms			MMF			
	n	Ave	SD	n	Ave	SD	
Total weight of eggs (g)	72	1233	1087	12	4180	1822	
Average weight of sinking eggs (g)	68	418.0	502.7	12	1908.3	673.5	
Average weight of floating (g)	67	892.5	786.7	12	2271.7	1369.0	
Average fertilization rate of eggs (%)	82	82.26	27.10	12	75.17	18.22	
Carotenoid							
Astaxanthin (AX, ng/egg)	89	0.4315	0.467	12	1.300	0.4690	
Fatty acid (%)							
ARA	92	1.5326	0.1616	12	2.2342	0.2139	
EPA	92	13.495	1.161	12	11.789	0.614	
DHA	92	28.834	2.385	12	27.746	1.620	
DHA/EPA	92	2.1577	0.3029	12	2.3533	0.627	
EPA/ARA	92	8.919	1.320	12	5.316	0.540	

The factors that were significantly different statistically ($P < 0.05$) are highlighted in yellow. Thus, the average number of eggs per batch and the average weights of sinking and floating eggs per batch were all significantly higher in the MMF groups. Egg batch weight has been shown to be related to size of the spawning female. MMF stock were slightly larger than VFF broodstock, however size differences cannot account for the magnitude of difference in mean egg batch size, and nutritional factors may be implicated. The figures could also be biased by the small number of egg batches analysed from MMF. It is also worthy of note that the % of floating eggs was higher in the VFF samples compared to the MMF samples (72 vs 54% respectively) while average fertilisation rate was not different between the two hatcheries. Differences in % floating eggs may be related to the rate and pattern of water flow within the tanks and through the egg collectors, which influences the recovery of sinking eggs within the tank.

The carotenoid pigment, astaxanthin (Ax) was significantly elevated in the MMF eggs compared to those from VFF with the former being 3-fold higher than the latter. Literature evidence suggests that increased egg carotenoid concentrations are linked to improved egg and larval quality in Atlantic cod (Salze et al., 2005; Sawanboonchun et al., 2008). The egg Ax concentrations reported here are lower (VFF) or intermediate (MMF) to the values reported by Sawanboonchun et al., (2008) in cod fed either an unsupplemented diet or one containing 80mg/kg Ax where the egg Ax concentrations were 1.0 and 2.7 ng/egg, respectively. The mechanism by which Ax exerts beneficial activity for egg and larval quality is not fully clarified but some possible explanations exist.

One explanation for the beneficial effects of Ax on cod egg quality could be that astaxanthin acts as a fertilisation hormone and improves fertilisation by stimulating and attracting spermatozoa (Hartmann et al., 1947). However, the ability of carotenoid pigments to absorb light and, thereby, quench or inactivate singlet oxygen and free radicals, is a more likely reason for their nutritional efficacy (Mayne, 1996). The mechanism by which the damaging effects of light, (UV and visible) and the subsequent generation of reactive oxygen species is attenuated, is a consequence of the conjugated polyene structure of carotenoids that allows sequestration and inactivation of these harmful molecules (Nishigaki et al., 1994). This action of carotenoids on control of damaging free radicals has lead to intervention studies in human conditions that have a pro-oxidant aetiology including heart disease, cancer, stroke, cataract, macular degeneration and immune modulation (Mayne, 1996). In natural spawning of cod, the eggs are released into the upper layers of the oceans, that are both highly illuminated and oxygen-rich, presenting an ideal environment for free radical generation. Thus, the improvements observed in egg and larval quality in farmed cod, when diets are supplemented with Ax, could be explained by better antioxidant protection both in the diet and in the eggs and larvae themselves (Cowey et al., 1985; Pangantihon-Kuhlmann et al., 1998).

The lipid analysis of the eggs showed that the MMF eggs were significantly higher in ARA, lower in EPA and the EPA/ARA ratio was subsequently lower compared to the VFF eggs (**Table 1**). There is a body of literature evidence showing concentration of ARA in egg lipids, compared to maternal lipids, in a range of fish species (Tocher and Sargent, 1984) and that increased egg ARA and reduced EPA/ARA ratio provides improvements in egg and larval quality in a number of fish species (Bruce et al., 1999; Mazorra et al., 2003). This might explain some of the improved parameters in egg quality observed in the present study but unfortunately this could not be substantiated by analysis of more samples from MMF, in the present study. For comparison, the HUFA content of the MMF moist diet and the VFF moist diet are shown in **Table 2.** This clearly shows the impact of dietary fatty acid compositions on egg compositions at the two hatcheries (**Tables 1 & 2).**

Table 2. A comparison of diet HUFA concentrations in moist broodstock diets collected from MMF and VFF in 2007 and 2008, respectively. Values are mean ± SD, n = 4.

Fatty acid (%)	MMF	VFF
ARA	3.19 ± 0.21	0.77 ± 0.44
EPA	6.31 ± 0.15	7.42 ± 0.04
DHA	10.45 ± 0.29	13.40 ± 0.04
EPA/ARA	1.98 ± 0.08	9.71 ± 0.19
DHA/EPA	1.68 ± 0.04	1.80 ± 0.01

The collection of egg samples from VFF was possible from 4/10/07 to 29/5/08 and this allowed a comparison of egg quality parameters and carotenoid and lipid concentrations between the different seasonal stocks spawning in autumn, spring and summer. This data is shown in **Table 3**.

Table 3. Seasonal variation in egg quality parameters, astaxanthin and essential fatty acid concentrations in eggs collected from Viking Fish Farms between October 2007 and May 2008.

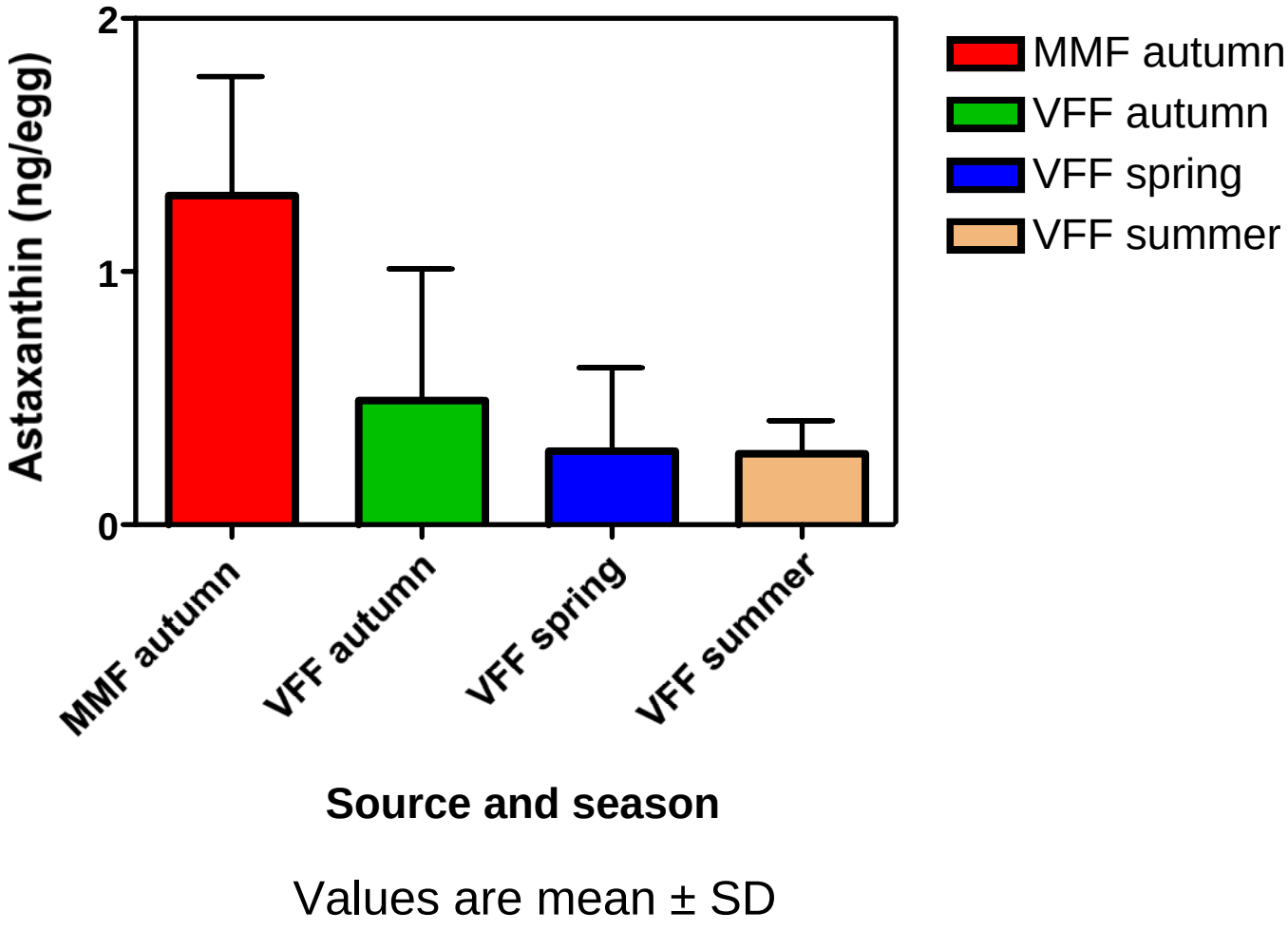
	Autumn			Spring			Summer			
	n	Ave	SD	n	Ave	SD	n	Ave	SD	
Total weight of eggs (g)	58	1308	1135	11	1095	851	3	285	58	
Average weight of sinking eggs (g)	55	485.5	535.0	10	138.1	131.5	3	113.7	60.5	
Average weight of floating eggs (g)	54	907.9	781.2	10	1025.8	865.6	3	171.3	56.5	
Average fertilization rate of eggs (%)	59	81.36	28.30	18	89.58	19.35	5	66.62	33.23	
Carotenoid										
AX (ng/egg)	62	0.4935	0.5143	21	0.2905	0.3300	6	0.2833	0.1329	
Fatty acid %										
ARA	65	1.4988	0.1662	21	1.6214	0.1219	6	1.5883	0.1025	
EPA	65	13.438	1.146	21	13.778	1.297	6	13.122	0.686	
DHA	65	28.800	2.550	21	29.385	1.794	6	27.277	1.798	
DHA/EPA	65	2.1637	0.3093	21	2.1610	0.3231	6	2.0817	1.411	
EPA/ARA	65	9.100	1.420	21	8.544	1.025	6	8.275	0.425	

Statistically significant values are highlighted in yellow. The results show a significantly increased number of sinking eggs recorded in the autumn spawners compared to the Spring and summer groups although the latter contained only a small number of egg batches. However, the percentage of floating eggs was highest in the Spring spawners at 94% with autumn spawners having 69% and summer spawners 60% of floating eggs. In terms of fatty acid compositions the highest concentrations of ARA were seen in the Spring spawners and the lowest in autumn spawners. Mean values for egg Ax concentration were highest in the autumn spawning fish, although there was considerable variation between batches across

all seasons. Ax concentrations in VFF autumn eggs were considerably lower than those seen in the MMF eggs (**Table 1).** The variation in the egg Ax concentrations between the three VFF groups and the MMF autumn eggs is also shown in **Figure 1.**

A comparison of the egg quality parameters and egg Ax and essential fatty acid concentrations across the eight broodstock tanks at VFF is shown in **Table 5**. Kruskal-Wallis analysis showed that the total weight of eggs produced was highest in tank R1 followed by R3 with lowest values in tanks F3 and RU2. Total weight of sinking eggs was highest in tank R1 followed by tank F2 with lowest values in tanks F1, F3 and RU2. Total weight of floating eggs was highest in tank R1 with similar values in tanks F1 and R3 and lowest values in tanks F3 and RU2. The highest fertilization rates were seen in tanks R3, F3 and F1 with lowest rates in tanks F2 and RU2. The highest egg astaxanthin concentrations were found in tank R1 followed by tank R2 and Big tank with lowest values in tanks R3 and F1. ARA values were highest in tanks R3 and F3 and lowest in tank R1 while EPA values were highest in tank R2 and lowest in F1. The EPA/ARA ratio was highest in tank R1 and lowest in tank R3.

Figure 1. Cod egg astaxanthin concentrations (ng Ax/egg) in Machrihanish Marine Farm autumn stocks and Viking Fish Farms autumn, spring and summer stocks.



In conclusion, tank R1 probably performed the best overall producing the highest total weight of eggs and floating eggs. The highest concentration of egg Ax was also seen in tank R1. Tank R3 had the highest level of egg ARA and, as a consequence had the lowest EPA/ARA ratio and these correlated with the highest fertilisation rate although the fertilisation rates for tanks F1 and F3 were very similar these were also correlated with high ARA and low EPA/ARA ratios. The increased fertilisation rate with increased egg ARA is in agreement with similar studies in other fish species (Bruce et al., 1999; Mazorra et al., 2003) while the improved egg production in R1 is in agreement with the results reported by Sawanboonchun et al (2008).

While improvements in broodstock performance may be influenced by individual nutrients it is also likely that broodstock source and genetics, season of sampling and photoperiod as well as diet and other environmental conditions also influence overall performance and these are all worthy of further study to elucidate their individual and collective roles in egg quality. Additional information on the individual tanks includes the following; The broodstock from **R1** were autumn spawners originating from Machrihanish and Ardtoe in 2004. The tank held 30 fish with recirculated and chilled water from early August. The average water temperature was 8^{o}C. These broodstock were fed a mix of Skretting Vitalis repro 17 mm and Dana 15 mm broodstock diet pellets.

The broodstock from Tank **R3** were wild fish from Millport and Loch Linnhe in 2002. There were seven fish from Millport and six from Loch Linnhe. They were held at ambient temperature and salinity and fed a sausage diet. The average weight of fish was 5.408 kg, length was 721 mm, on 22 August 2007. Tank **R3** spawned in spring.

The broodstock from Tank **R2** came from Ardtoe and spawned in spring. They were originally wild fish from Orkney PP in 2002. There were 57 fish held under ambient photperiod and the broodstock were fed Bioma 12 mm, Skretting co Europa 13 mm and Skretting Vitalis 17 mm diet. The average weight of broodstock was 4.013 kg and length 648 mm measured on 11 May 2006

The broodstock from Tank **F1** spawned in Autumn and the stock were from the wild (Ireland and Aultbea). Age at first spawning was not known. There were 10 fish from Ireland and 7 fish from Aultbea. The water was not chilled until 11/9/07 and the broodstock were fed marine sausage diet.

The astaxanthin content of eggs form the different broodstock tanks at Viking Fish Farms is shown in **Figure 2.** The lowest concentration of Ax was 0.1 ng/egg in tank R3 and the highest was 1.1 ng/egg in tank R1. It is not entirely clear why there is such a large variation between tanks but it is likely due to the different dietary regimes that were used at VFF. The value of 1.1 ng/egg in tank R1 is close to the average value of 1.3 ng/egg seen in the MMF fish, although the VFF average of 0.39 ng/egg was considerably lower than the MMF average. Given the importance of Ax for good egg quality it would be good practice to set an egg Ax concentration of ~1ng/egg as a possible target value (Sawanboonchun et al., 2008).

Figure 2. Astaxanthin content of eggs (ng/egg) from different broodstock tanks at Viking Fish Farms

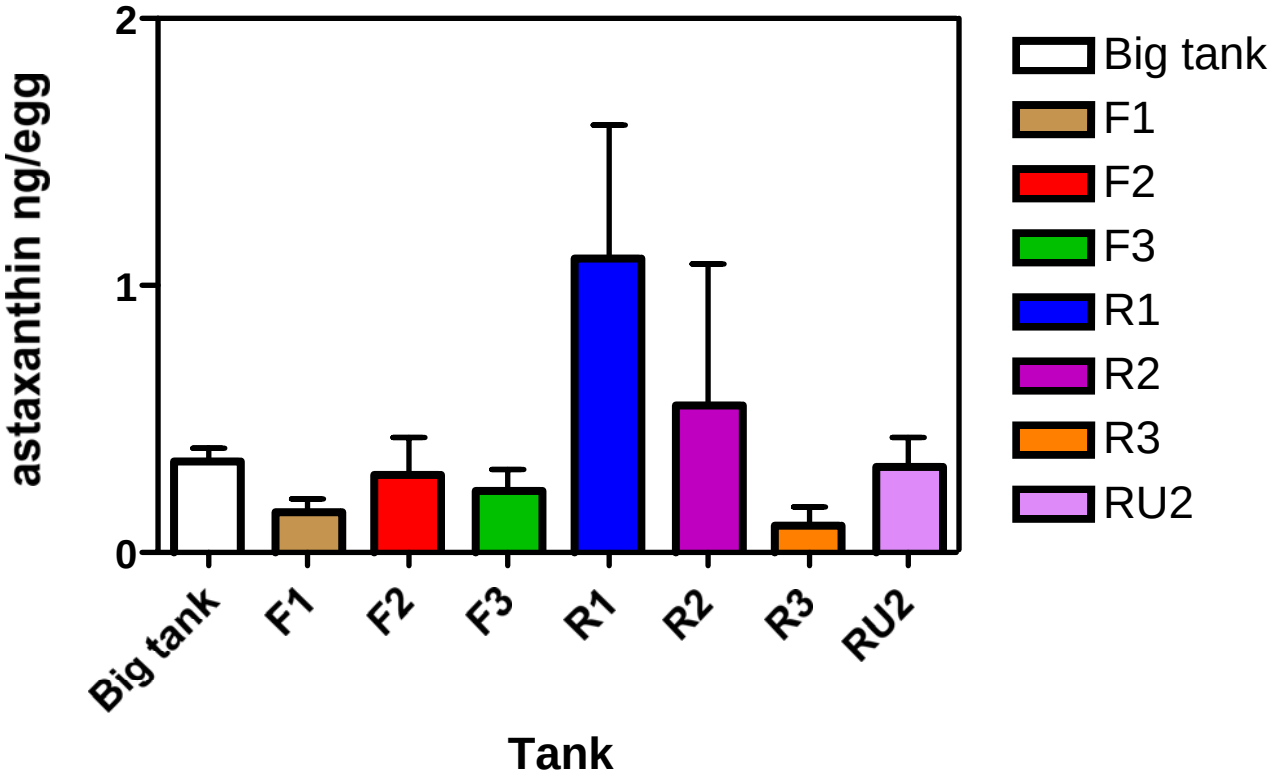


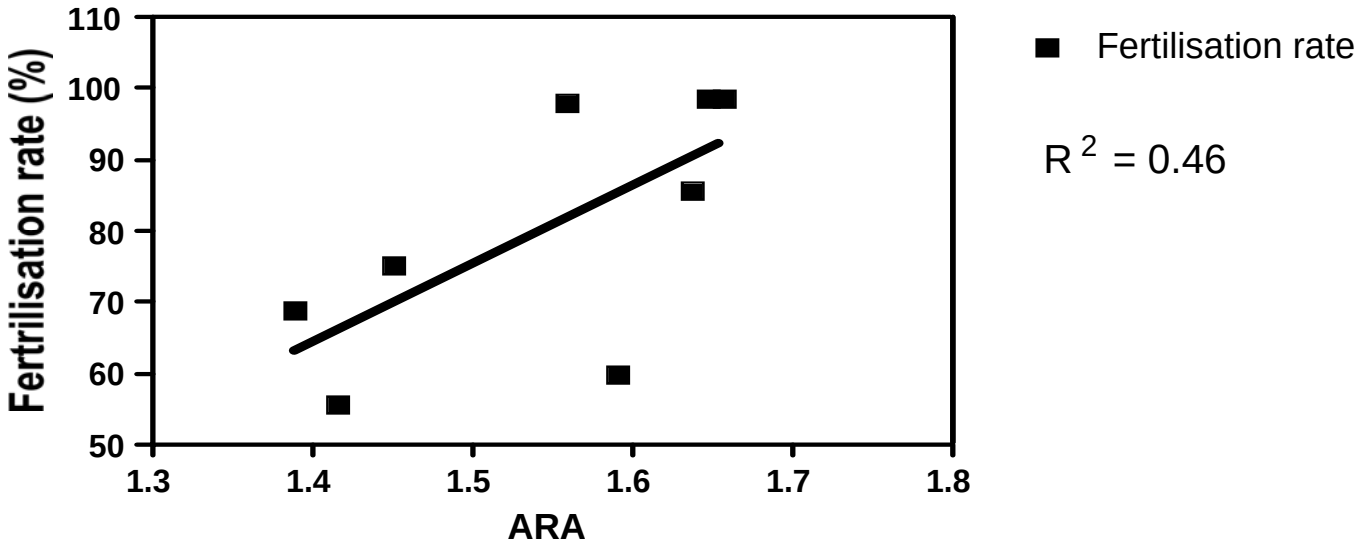
Table 5. A comparison of egg quality, astaxanthin and essential fatty acid concentration in individual broodstock tanks at Viking Marine Farms from 4/10/07 to 29/5/08. Values that are significantly different are shown in yellow highlight.

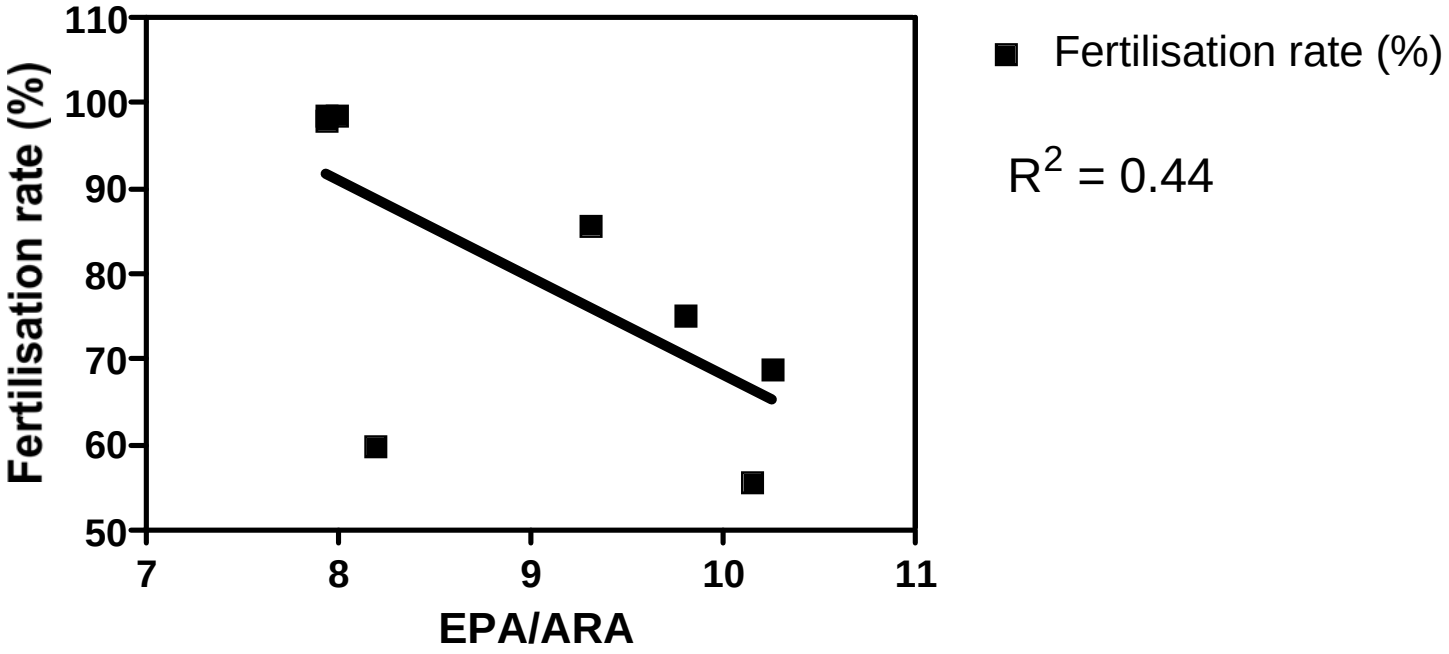
Tank	Big Tank			F1			F2			F3		
	n	Ave	SD	n	Ave	SD	n	Ave	SD	n	Ave	SD
Total weight of eggs (g)				14	1055.2	935.8	5	961.2	931.3	14	356.9	247.9
Average weight of sinking eggs (g)				14	107.5	110.2	5	365.0	207.4	11	106.1	49.8
Average weight of floating eggs (g)				12	1108.6	832.4	5	596.2	786.3	12	304.4	216.7
Average fertilization rate of eggs (%)	4	75.25	31.26	14	98.05	2.97	8	55.66	31.45	12	98.58	1.93
Carotenoid												
AX(ng/egg)	5	0.34	0.0548	17	0.1529	0.0514	8	0.2875	0.1458	15	0.2333	0.0816
Fatty acid %												
ARA	5	1.45	0.0982	17	1.5576	0.1424	8	1.415	0.1235	15	1.6473	0.1287
EPA	5	14.166	0.335	17	12.260	0.611	8	14.230	0.984	15	13.055	0.897
DHA	5	28.368	0.850	17	31.606	1.604	8	28.074	2.195	15	28.969	1.433
DHA/EPA	5	2.0020	0.0335	17	2.5835	0.1745	8	1.9913	0.3173	15	2.2273	0.1457
EPA/ARA	5	9.804	0.680	17	7.938	0.862	8	10.151	1.300	15	7.993	1.105

Tank	R1			R2			R3			RU2		
	n	Ave	SD	n	Ave	SD	n	Ave	SD	n	Ave	SD
Total weight of eggs (g)	23	2087.6	1142.3				13	1179.2	837.0	3	285.0	57.7
Average weight of sinking eggs (g)	23	952.7	529.9				12	139.7	127.1	3	113.7	60.5
Average weight of floating eggs (g)	23	1134.7	786.2				12	1104.1	854.6	3	171.3	56.5
Average fertilization rate of eggs (%)	23	69.09	31.77	5	85.84	21.13	12	98.75	2.80	4	60.10	34.49
Carotenoid												
AX (ng/egg)	20	1.1050	0.4925	6	0.5500	0.5320	13	0.1000	0.0707	5	0.3200	0.1095
Fatty acid %												
ARA	23	1.3887	0.1159	6	1.6367	0.1003	13	1.6562	0.1181	5	1.5900	0.1145
EPA	23	14.196	0.937	6	15.207	0.784	13	13.072	0.720	5	12.984	0.668
DHA	23	26.972	2.193	6	26.880	1.022	13	30.322	1.175	5	27.740	1.558
DHA/EPA	23	1.8991	0.0850	6	1.7700	0.0477	13	2.3277	0.1725	5	2.1360	0.0522
EPA/ARA	23	10.248	0.524	6	9.307	0.497	13	7.927	0.684	5	8.182	0.401

Plots were made of fertilisation rate against egg ARA content and the egg EPA/ARA ratio (**Figure 3 A and 3B**). These showed that fertilisation rate was correlated with both egg ARA and EPA/ARA ratio (R^2 = 0.46 and 0.44, respectively). No correlations were found between Ax and fertilisation rate, total egg production, floating egg production or ARA and total egg production or total floating egg production.

Figure 3A and 3B. Plot of egg ARA versus fertilsation rate (%) and EPA/ARA ratio versus fertilisation rate (%) in the eight broodstock tanks of Viking Fish Farms.





Maternal blood lipid compositions as predictors of egg fatty acid compositions

The final stage of the project was to investigate whether maternal blood lipids could be used as predictors of egg fatty acid concentrations and ratios. Therefore, samples of blood and eggs, obtained by hand stripping, were collected from 32 broodstock at MMF in February and March 2008. The blood was separated into red blood cell (RBC) and plasma fractions by centrifugation and the eggs, RBC and plasma fatty acid compositions were determined. A summary table of the main essential HUFA and their ratios in the 2 blood fractions and eggs are shown in **Table 6**. ARA levels were lower in eggs compared to both blood fractions while RBC had higher EPA but lower DHA than eggs. Plasma fatty acids tended to be more similar to egg values. These differences in blood and egg lipids probably reflect the relative abundance of neutral and polar lipids in the blood fractions where the polar lipids tend to accumulate more HUFA than the neutral lipids.

Table 6. Concentrations of arachidonic, eicosapentaenoic and docosahexaenoic acids in unfertilised eggs, red blood cells and plasma in Atlantic cod broodstock. Values are weight % of total fatty acids, n =32.

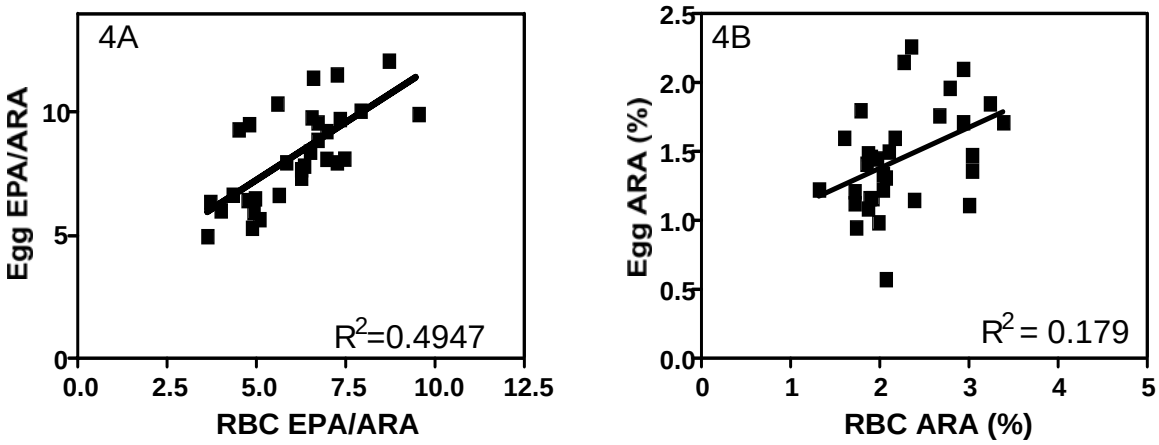
	Unfertilised eggs			Red blood cells			Plasma		
	n	Ave	SD	n	Ave	SD	n	Ave	SD
Fatty acid									
ARA	32	1.45	0.35	32	2.23	0.54	32	2.07	0.75
EPA	32	11.63	0.96	32	12.96	1.17	32	11.15	0.56
DHA	32	25.83	2.05	32	23.46	1.77	32	25.84	1.60
DHA/EPA	32	2.22	0.11	32	1.82	0.20	32	2.32	0.15
EPA/ARA	32	8.22	1.88	32	6.10	1.42	32	6.04	1.99

Plots of egg and blood fraction HUFA % were analysed using linear regression to establish whether egg HUFA could be correlated with blood lipids. The results of these analyses are shown in **Table 7.** Generally, R^2 values were quite low suggesting only weak correlation between egg and blood HUFA. The R^2 values ranged from 0.016 for RBC EPA to 0.495 for RBC EPA/ARA ratio while values for ARA and DHA/EPA ratio in eggs versus blood gave R^2 values from 0.151 to 0.214. These data suggest that the EPA/ARA ratio in RBC could be used as a fairly accurate predictor of the EPA/ARA ratio in the eggs. The blood ARA concentrations were better indicators of egg ARA than similar associations between EPA and DHA. The plots of EPA/ARA ratio in eggs and red blood cells and egg ARA and RBC ARA are shown in **Figures 4A and 4B.**

Table 7. R^2 values for regression analysis of HUFA and HUFA ratios in egg lipids versus red blood cell and plasma lipids in Atlantic cod broodstock.

Egg fatty acid	vs	Blood fatty acid	R^2 value
ARA		Plasma ARA	0.156
ARA		RBC ARA	0.179
EPA		Plasma EPA	0.027
EPA		RBC EPA	0.016
DHA		Plasma DHA	0.080
DHA		RBC DHA	0.039
EPA/ARA		Plasma EPA/ARA	0.257
EPA/ARA		RBC EPA/ARA	0.495
DHA/EPA		Plasma EPA/ARA	0.151
DHA/EPA		RBC DHA/EPA	0.214

Figures 4A & 4B.



References

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