

A pigmentation model for farmed Atlantic salmon: Nonlinear regression analysis of published experimental data

Odd Inge Forsberg^a, Atle G. Guttormsen^{b,*}

^a Integral Aquaculture Ltd., Norway

^b Norwegian University of Life Sciences, Department of Economics and Resource Management, P.O. Box 5003, N-1432 Ås, Norway

Received 29 March 2005; received in revised form 29 August 2005; accepted 1 September 2005

Abstract

The most prominent quality characteristic of post-smolt Atlantic salmon is the red/pink flesh colour. The primary source of this colouration is caused by deposition of relatively large amounts of colour pigments, such as astaxanthin and canthaxanthin, obtained from their diet. The accumulation of astaxanthin in the muscle is also dependent on fish size. The fish farmer must therefore optimise the dietary composition supplied to salmon that results in the desired flesh colour required by the customers. This study modelled the relationships between chemical muscle astaxanthin concentration and visual colour perception (*SalmoFan* score), and the explanatory variables dietary astaxanthin concentration and fish size. Parameter estimates were analyzed by nonlinear regression analysis (Levenberg–Marquardt algorithm). The results showed that the two dependent variables gave excellent fit to the observed data ($R^2=0.968$; $N=43$). The mathematical model developed in this study can therefore be used to explore the relationship flesh colour in salmon and its relationship to fish size and dietary composition on an integrated way.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Muscle astaxanthin; *SalmoFan* score; Atlantic salmon; Statistical analysis

1. Introduction

Farming of Atlantic salmon (*Salmo salar* L.) is an emerging global industry with an annual production of approximately 1,200,000 metric tons in year 2003, representing a market value of 4.3 billion USD or 3.8 billion Euros. About 83% of farmed salmon is produced in Norway, Chile and the UK, and fish are distributed into the global markets; each with different requirements regarding to fish quality.

The primary source of this colouration in salmon is caused by deposition of relatively large amounts of carotenoids, such as astaxanthin and canthaxanthin, obtained from their diet (Torrisen, 1995; Torrisen et al., 1995). Studies have shown that astaxanthin concentration in Atlantic salmon muscle can also be controlled by other factors, such as dietary fat content (Bjerkeng et al., 1997; Einen and Roem, 1997) and fish size (Akvaforsk, unpublished; Mørkøre and Rørvik, 2001). In commercial farming practice, dietary colour pigments comprises about 15–20% of the total feed cost, or 6–8% of production costs (Torrisen, 1995). For salmon customers however, the visual perception of fillet redness is more important than chemical astaxanthin analysis or from indirect measurements

* Corresponding author.

E-mail addresses: odd-fors@online.no (O.I. Forsberg), atle.guttormsen@umb.no (A.G. Guttormsen).

(Alfnes et al., 2005). Visual colour assessment is aided by the use of colour charts, such as *Roche Colour Card* for Salmonids and the *SalmoFan* (Hoffmann-La Roche, Basel, Switzerland) (Skrede et al., 1990). The *SalmoFan* score (*SF* score) is now the most frequently used tool to assess the visual fillet colour in Atlantic salmon. Studies have found a significant linear correlation between *SalmoFan* score and muscle astaxanthin concentration (Christiansen et al., 1995). This relationship is an important bridge between biological experiments studying astaxanthin deposition in fish, and market/customer studies where visual perception of flesh colour is of interest.

One of the most important operational processes in commercial fish farms is therefore to balance fish astaxanthin concentration to the customer demand at minimum cost. The fish farmer's major tool is to balance the diet composition supplied to salmon that results in the desired product quality. The aim of this study is to build a mathematical model that can be used to optimise the dietary astaxanthin concentration in the seawater period until fish has reached market size. The model can thus be used as a decision support which may contribute to reduce production costs in salmon farming. This paper presents a mathematical model describing the relationships between dietary pigmentation concentration and fish size on muscle astaxanthin concentration in post-smolt Atlantic salmon and its resulting *SalmoFan* score.

2. Materials and methods

2.1. Data set for analysis

Data were compiled from six Norwegian experimental studies with post-smolt Atlantic salmon performed in the period 1993–2002 (Torrissen et al., 1995; Bjerkeng et al., 1997; Einen and Roem, 1997; Akvaforsk, unpublished, Buttle et al., 2001, Hamre et al., 2004); each with its unique set of experimental conditions (Table 1). Each of the trials contained complete data set ($N=43$) for fish astaxanthin concentration (A_f , mg/kg fish) and the independent variables dietary astaxanthin concentration (A_d) ranging between 0 and 200 mg/kg diet, and fish size (W) ranging between 0.5 and 5.0 kg. Water temperature in the various trials ranged from 4 to 15 °C, and fish were fed to satiation with high-energy diets (23–39% fat) in all trials. The data set was used for nonlinear statistical regression analysis.

2.2. Muscle astaxanthin model

The dose–response relationship between dietary and muscle astaxanthin concentration presented in Torrissen et al. (1995), can be described from Michaelis–Menton kinetics:

$$A_f = \frac{K_m A_d}{c + A_d} \quad (1)$$

Table 1
Data compiled from the seven studies used in the statistical analysis ($N=43$)

Fish size (kg)	Dietary astaxanthin (mg/kg feed)	Dietary fat (% in diet)	Muscle astaxanthin (mg/kg feed)	Sample method ^a	N	Notes	References
2.5–2.7	45–52	30–39	6.1–7.3	A	4	Pooled data from both fish meal and soybean meal groups	Bjerkeng et al., 1997
1.5–3.3	55–67	25–39	5.5–7.6	B	6	Fish response on quality traits from varying dietary fat content. Data were pooled from Groups 1 and 2	Einen and Roem, 1997
3.2	0–200	23	0–8.0	A	9	Dose–response trial	Torrissen et al., 1995
1–6	60	28–30	5.5–7.5	B	5	Data compiled from unpublished experiments	Akvaforsk, unpublished
0.8	60	26	2.3–2.4	A	3	Data set G1, G2, G3 were used only	Buttle et al., 2001
0.5–0.8	10 and 50	6.5 and 32	1.3–2.8	A	16	Data set for week 23 were used only	Hamre et al., 2004

A: Whole fillet samples. Sample of 10–20 fish. Whole fillet was minced and blended. Sub-sample were taken from the blend and analyzed by HPLC.
B: Norwegian Quality Cut (NQC) samples. Sample of 10 fish. Cutlet was taken posterior to the base of the dorsal fin; was minced and blended. Sub-sample were taken from this blend and analyzed by HPLC.

^a Two different sampling methods were used.

Table 2

Parameter estimates for the pigmentation model (Eq. (4)) using the Levenberg–Marquardt algorithm

Parameter	Estimate mean (S.E.)	Summary statistics
a	11.1 (0.4)	$N=43$
b	-0.829 (0.045)	$R^2=0.968$
c	11.9 (2.1)	R^2 (adjusted for df)=0.955
d_B	0.26 (0.18)	Standard error of estimate: 0.480 Mean absolute error: 0.350 Durbin–Watson statistics: 1.459

where K_m =maximum saturation of astaxanthin in fish muscle, and c =half-saturation constant (concentration of A_d which results in $A_f=K_m/2$). Data from Akvaforsk (unpublished) show that fish fed high astaxanthin concentration diets (60 mg/kg), had a size-dependent response in astaxanthin deposition:

$$A_{f_{\max}} = a \cdot \exp^{-b/W} \quad (2)$$

We then assume that K_m equals the $A_{f_{\max}}$ expression in Eq. (2). Model (3) describes the relationships between muscle astaxanthin concentration and the explanatory variables dietary astaxanthin and fish size as:

$$A_f = a \cdot \exp^{-b/W} \frac{A_d}{c + A_d} \quad (3)$$

where the coefficient values a , b , and c will be estimated. Due to systematic variations in model outputs between the six trials due to differences in methods of sampling and measurement of fish astaxanthin (see Table 1), we have to adjust model (3) for these errors. For each data set, we include a binary dummy variable x_B , where $x_B=1$ if the actual trial was analyzing fillets from the Norwegian Quality Cut (NQC) only; (Method B; see Table 1), otherwise $x_B=0$. Then, the model (4) is adjusted for systematically sampling differences:

$$A_f = a \cdot \exp^{-b/W} \frac{A_d}{c + A_d} + x_B d_B \quad (4)$$

where d_B is a model coefficients representing the positive (or negative) deviation of samples taken from NQC fillets compared to whole fish analysis.

2.3. Parameter estimation

The Levenberg–Marquardt (LM) algorithm was used for parameter estimation of a , b , c and d_B . The LM algorithm is an iterative technique that locates the minimum of a multivariate function that is expressed as the sum of squares of nonlinear real-valued functions

(Marquardt, 1963). It has become a standard technique for nonlinear least-squares problems widely adopted in a broad spectrum of disciplines. The software package STATGRAPHICS 5.0 Professional (Manugistics Inc. Rockville, MD, USA, www.statgraphics.com) was used for the nonlinear regression analysis.

The results from the estimation of parameter values are presented in Table 2.

3. Model results

3.1. Parameter estimates

Model (4) showed an extraordinary good fit to measured data, and dietary astaxanthin and fish size explained 96.8% of the variances in the flesh astaxanthin concentration in salmon (Fig. 1). Estimates of d_B demonstrate that astaxanthin concentrations from NQC fillets was systematically 0.26 mg/kg higher than whole fish analysis. Inclusion of both water temperature and dietary fat content as explanatory variables did not increase R^2 values.

Model (4) gives also an estimate for maximum saturation of astaxanthin in fish muscle: $K_m = a \cdot \exp^{-b/W}$. As presented in Fig. 2, values of K_m increase curve-linearly with increasing fish size, and converge towards the a -value ($a=11$ mg astaxanthin/kg fish).

3.2. Model of visual expression of flesh redness

Visual colour assessments may be aided by the use of colour charts such as the Roche Colour Card for Salmonids (RCC) and the SalmoFan (SF) (Hoffmann-

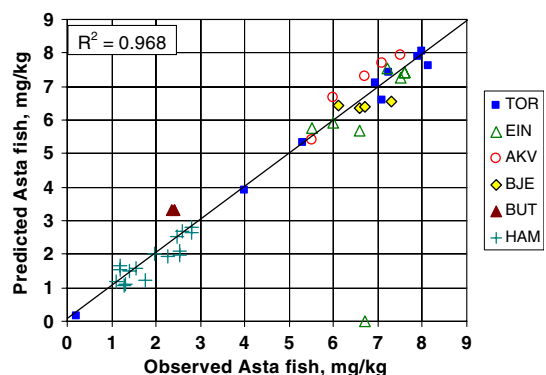


Fig. 1. Observed vs. predicted astaxanthin concentrations in Atlantic salmon fillet. Observed data are compiled from six experiments: TOR (Torrisen et al., 1995), EIN (Einen and Roem, 1997), AKV (Akvaforsk, unpublished), BJE (Bjerkeng et al., 1997), BUT (Buttle et al., 2001), HAM (Hamre et al., 2004). Predictions are based on Eq. (4) inserted data from the above studies. $N=43$.

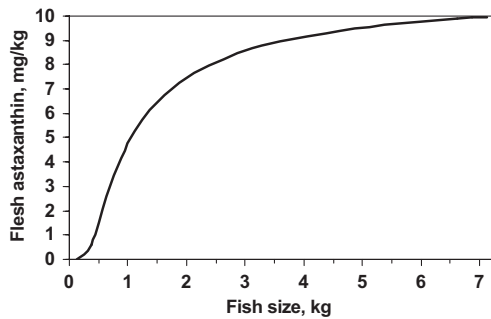


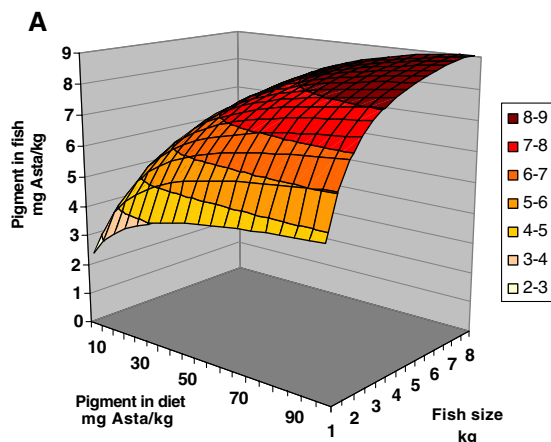
Fig. 2. Astaxanthin saturation constant (K_m) in Atlantic salmon muscle in varying fish sizes. Graph was estimated from Eq. (2).

La Roche, Basel, Switzerland). Such assessments represent crude measures to characterize and classify pigmentation of fish flesh, primarily for the use by fish farmers, fish feed producers, processing industry and marketing organizations. SF is a further development of the RCC, and has today replaced the RCC as a globally accepted tool for evaluation and communication of salmonid flesh colour. The *SalmoFan* score card range over 14 red colours with varying intensity of redness. Each of the colours is associated with a numerical value ranging from 20 (very pale red) to 34 (very intense red). The relation between RCC and SF score is:

$$SF = 2RCC - 3. \quad (5)$$

Christiansen et al. (1995) found a strong linear relationship between RCC scores and salmon astaxanthin concentration, A_f : ($N=540$, $R^2=0.992$):

$$A_f = 1.34RCC - 12.67 \quad (6)$$



Combining Eqs. (5) and (6) we have a linear relationship between SF score and A_f can be established:

$$SF = 1.49A_f + 15.91 \quad (7)$$

Inserting Eq. (3), inserted estimates of model coefficients a , b and c into (7) gives an estimate for whole fish (SF_w) and NQC (SF_{NQC}) as:

$$SF_w = 16.48 \cdot \exp^{-0.829/W} \frac{A_d}{11.9 + A_d} + 15.91 \quad (8)$$

$$SF_{NQC} = 16.48 \cdot \exp^{-0.829/W} \frac{A_d}{11.9 + A_d} + 16.17 \quad (9)$$

Models (8) and (9) assume that fish are supplied feed with a constant pigment concentration throughout its life cycle. These models do also assume that no seasonal differences in astaxanthin dose–response relationship and its fish size dependency are present.

Fig. 3 presents graphically the relationships between fish size and dietary astaxanthin concentration on fillet astaxanthin concentration and the corresponding *SalmoFan* score. The surface-plots show clearly that astaxanthin concentration increases strongly and curve-linearly with increasing fish size and dietary astaxanthin concentration. The model assumes that maximum fish astaxanthin concentration are 11 mg/kg fillet, and maximum *SalmoFan* score values is 29.5 for 8 kg fish fed 100 ppm astaxanthin throughout the growth period.

3.3. Dynamical pigment model

Eqs. (4) and (8–9) can be applicable for modelling fish muscle astaxanthin concentration, and the resulting

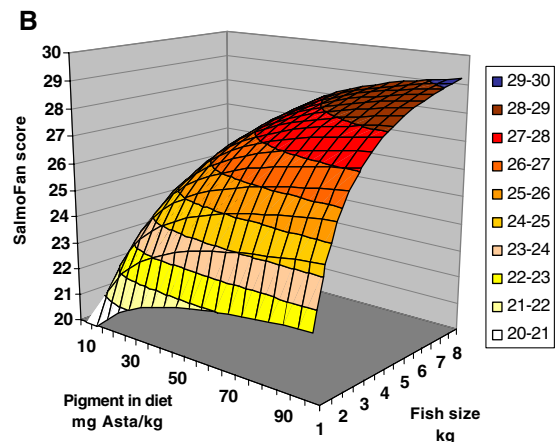


Fig. 3. The relationship between fish size and dietary astaxanthin content on whole fish flesh astaxanthin concentration (A) and *SalmoFan* score (B) in farmed Atlantic salmon. The models are based on Eqs. (4) and (9), respectively.

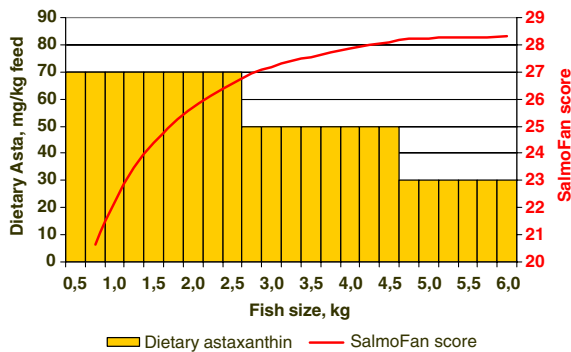


Fig. 4. *SalmoFan* score (whole fish average) for Atlantic salmon estimated from model (10) for varying fish sizes (solid line). Dietary inputs (bars) were collected from a commercial feed table.

SalmoFan score, in farmed Atlantic salmon throughout its growth period from seawater transfer to time of harvest. However, the model can only be applied in cases where the dietary composition is kept constant over the seawater growth period. This is normally not the case. In commercial salmon diets, astaxanthin concentration tends to decrease when fish grow. For salmon fed to satiation, the difference Eq. (10) can therefore be applied to estimate astaxanthin concentration in salmon muscle at time T :

$$A_{f_t} = \frac{1}{W_t} \left(W_1 A_{f_1} + \sum_{t=1}^T \frac{W_{t+1} A_{f_{t+1}} - W_t A_{f_t}}{W_{t+1} - W_t} \right) \quad (10)$$

where W_t = fish size at time t and A_{f_t} = fish astaxanthin concentration at t estimated by inserting dietary astaxanthin (A_d) used in period $(t, t+1)$ into Eq. (4). Example of the model is presented in Fig. 4.

4. Discussion

The dose–response relationship between dietary astaxanthin and muscle pigmentation shows a clear diminishing yield of astaxanthin with increasing dietary dose in Atlantic salmon (Torrisen et al., 1995) and rainbow trout (Bjerkeng et al., 1990; Storebakken and No, 1992). Similar relationships have been demonstrated for both dietary astaxanthin and canthaxanthin. For both species, little extra flesh pigmentation is gained by using doses higher than ca. 50–60 mg/kg in diet. Generally, dose–response relationships are in principle valid only for a specific dose formulation, under specific experimental conditions, for a specific species of a specific physiological condition and size. For example, the fish in the experiment reported by Torrisen et al. (1995) had low growth (used twice as long time to reach 3–4 kg compared to Atlantic salmon in commercial

farming of today), and were subject to four drug treatments towards furunculosis disease. There is therefore a need for documentation of the dose–response on fast growing and well-performing Atlantic salmon to optimise the dietary doses.

The dietary response on salmon pigmentation is dependent on the fish size. Salmonids with access to astaxanthin containing diets accumulate carotenoids as they grow, and accumulation of muscle carotenoids will not cease until the fish start to become sexually mature (Bjerkeng et al., 1992). Deposition of carotenoids in the muscle is slow in young salmon and increase with increasing fish size (Bjerkeng et al., 1992). As a result, the concentration of fillet astaxanthin normally increases with increasing fish size, which is consistent with the results from this study. As a result, the astaxanthin saturation constant in salmon flesh is size dependent which is presented in Fig. 2.

There is evidence that other dietary factors than dietary pigment content has an impact on fish astaxanthin concentrations. Among the dietary macronutrients, lipids appear to affect pigmentation appreciably. It is also shown that both dietary fatty acid composition (Waagbø et al., 1993; Bjerkeng et al., 1999), and dietary lipid content may influence astaxanthin deposition in salmonids. Astaxanthin deposition and retention was higher both in rainbow trout (Torrisen, 1985; Nickell and Bromage, 1998) and in Atlantic salmon (Einen and Roem, 1997; Bjerkeng et al., 1997; Hamre et al., 2004) fed increasing amounts of dietary lipids. These findings were however not identified in this statistical analysis.

In the data set included in our statistical analysis, both whole fish and Norwegian Quality Cut (NQC) cutlets were analyzed. It may lead to biases when data from studies with different sampling procedures were pooled and analyzed statistically. The reason for that is that astaxanthin is not evenly distributed within the fish fillets. A longitudinal variation in carotenoid content and red colour is found, with more astaxanthin being deposited in the caudal (tail) than in the anterior (front) part (No and Storebakken, 1991). Pigment analysis of whole fish and in the NQC may therefore give different results. In this study we have demonstrated, however, that the use of dummy variables in the multiple regression analysis may adjust for systematically differences in measurement methods. Our analysis show that the astaxanthin concentration in NQC fillets are, on average, is 0.26 mg/kg fish higher than minced whole fillet analysis, which is reasonable findings since NQC is located at the posterior, more colourful, part of the fish.

In this study, parameter estimates were drawn from analyses of the small-scale, controlled experiments, which may not fully represent conditions in commercial grow out farms. Studies have shown that there are other than fish size and dietary factors that may determine the astaxanthin deposition and visual colour in fish flesh. Among these are genetic origin (Rye and Gjerde, 1996; Gjedrem, 2000), stress level prior to slaughtering (Robb et al., 2000; Sigholt et al., 1997) and temperature after slaughtering (Torrissen et al., 1999). There is also evidence that time of seawater transfer (0+, 1+ smolts) and seasonality may have an impact on salmon flesh colour (Mørkøre and Rørvik, 2001). The pigmentation models should therefore be used with cautious, and should be validated against commercial farming data before they are used for managerial decision making.

Acknowledgements

We kindly thank the Institute of Aquaculture Research (AKVAFORSK), Norway, for permission to use their data from unpublished experiments.

References

- Alfnes, F., Guttormsen, A.G., Steine, G., Kolstad, K., 2005. Consumers' willingness to pay for the color of salmon: a choice experiment with real economic incentives. Selected paper American Association of Agricultural Economics, 2005 Annual Meeting, July 24–27, 2005, Providence, Rhode Island.
- Bjerkeng, B., Storebakken, T., Liaen-Jensen, S., 1990. Response to carotenoids by rainbow trout in the sea: resorption and metabolism of dietary astaxanthin and canthaxanthin. *Aquaculture* 91, 153–162.
- Bjerkeng, B., Storebakken, T., Liaen-Jensen, S., 1992. Pigmentation of rainbow trout from start feeding to sexual maturation. *Aquaculture* 108, 333–346.
- Bjerkeng, B., Refstie, S., Fjalestad, K.T., Storebakken, T., Rødbotten, M., Roem, A., 1997. Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. *Aquaculture* 157, 297–309.
- Bjerkeng, B., Hatlen, B., Wathne, E., 1999. Deposition of astaxanthin in fillets of Atlantic salmon (*Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils. *Aquaculture* 180, 307–319.
- Buttle, L.G., Crampton, V.O., Williams, P.D., 2001. The effect of feed pigment type on flesh pigment deposition and colour in farmed Atlantic salmon *Salmo salar* L. *Aquac. Res.* 32, 103–111.
- Christiansen, R., Struksnæs, G., Estermann, R., Torrissen, O.J., 1995. Assessment of flesh colour in Atlantic salmon, *Salmo salar* L. *Aquac. Res.* 26, 311–321.
- Einen, O., Roem, A.J., 1997. Dietary protein/energy ratios for Atlantic salmon in relation to fish size: growth, feed utilization and slaughter quality. *Aquac. Nutr.* 3, 115–126.
- Gjedrem, T., 2000. Genetic improvement of cold-water fish species. *Aquac. Res.* 31, 25–33.
- Hamre, K., Christiansen, R., Waagbø, R., Maage, A., Torseinsen, B.E., Lygren, B., Lie, Ø., Albrektsen, S., 2004. Antioxidant vitamins, minerals and lipid levels in diets for Atlantic salmon (*Salmo salar* L.): effects on growth performance and fillet quality. *Aquac. Nutr.* 10, 113–123.
- Marquardt, D.W., 1963. An algorithm for the least-squares estimation of nonlinear parameters. *SIAM J. Appl. Math.* 11 (2), 431–441.
- Mørkøre, T., Rørvik, K.-A., 2001. Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (*Salmo salar* L.) transferred to seawater as 0+ smolts or 1+ smolts. *Aquaculture* 199, 145–157.
- Nickell, D.C., Bromage, N.R., 1998. The effect of dietary lipid level on variation of flesh pigmentation in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 161, 237–251.
- No, H.K., Storebakken, T., 1991. Colour stability of rainbow trout fillets during frozen storage. *J. Food Sci.* 56, 969–972, 984.
- Robb, D.H.F., Kestin, S.C., Warriss, P.D., 2000. Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. *Aquaculture* 182, 261–269.
- Rye, M., Gjerde, B., 1996. Phenotypic and genetic parameters of body composition traits and flesh colour in Atlantic salmon, *Salmo salar* L. *Aquac. Res.* 27, 121–133.
- Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T., Seland, A., 1997. Handling stress and storage temperature affect meat quality of farm-raised Atlantic salmon (*Salmo salar*). *J. Food Sci.* 62, 898–905.
- Skrede, G., Risvik, E., Huber, M., Enersen, G., Blümlein, L., 1990. Developing a colour card for raw flesh of astaxanthin-fed salmon. *J. Food Sci.* 55, 361–363.
- Storebakken, T., No, H.K., 1992. Pigmentation of rainbow trout. *Aquaculture* 100, 209–229.
- Torrissen, O.J., 1985. Pigmentation of salmonids: factors affecting carotenoid deposition in rainbow trout (*Salmo gairdneri*). *Aquaculture* 46, 133–142.
- Torrissen, O.J., 1995. Strategies for salmonid pigmentation. *J. Appl. Ichthyol.* 11, 276–281.
- Torrissen, O.J., Christiansen, R., Struksnæs, G., Estermann, R., 1995. Astaxanthin deposition in the flesh of Atlantic salmon, *Salmo salar* L., in relation to dietary astaxanthin concentration and feeding period. *Aquac. Nutr.* 1, 77–84.
- Torrissen, O.J., Roth, G., Slinde, E., 1999. Effects of storage and slaughter temperature on fillet colour of Atlantic salmon (*Salmo salar*). Poster at the Farmed Fish Quality Conference, April 1999, Bristol, England.
- Waagbø, R., Sandnes, K., Torrissen, O.J., Sandvin, A., Lie, Ø., 1993. Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of N-3 polyunsaturated fatty acids at two levels of vitamin E. *Food Chem.* 46, 361–366.