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SEXUAL MATURATION: EFFECT ON PROTEASE ACTIVITIES AND CAROTENOID LEVELS IN ATLANTIC SALMON (Salmo salar)

by

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ABSTRACT

Protease activities in the digestive tract and carotenoid levels in flesh, ovaries and plasma were determined in 4 to 4 1/2 year old Atlantic salmon (Salmo salar) during the period from early March until December.

The protease activities in the digestive tissue significantly changed during the maturation process. The sexual maturation significantly decreased the level of astaxanthin in the flesh and ovaries, but the total amount in the ovaries still increased. The covariance between astaxanthin in plasma and tryptic-like activities in the digestive tissues were significant, and the level of astaxanthin in plasma was also influenced by time and stage of sexual maturation.

INTRODUCTION

The maturation of anadromous salmonids involves a significant change in the metabolism. During the relatively short period of time, from April to November-December, the gonads increase in size from less than 1% of body weight to about 20-25%, mainly during a period when the fish do not feed. The growth of gonads during the late period of maturation is therefore a result of mobilization of the body resources.

Mobilization of the flesh carotenoids and deposition of it in the skin and ovaries during the maturation of various salmonid species are reported and also quantified by several authors, (Steven, 1949; Crozier, 1970; Sivetseva and Dubrovin, 1981; Kitahara, 1983). This depletion of flesh pigments has important economical aspects by being one of the factors limiting the acceptability of maturing salmonids by the consumers. This change in distribution of carotenoids is also the basis of the hypothesis that carotenoids have a function in reproduction (Tacon, 1981; Torrissen, 1984b).

Salmonids have a relatively high protein requirement. This, together with the fact that the salmonids stop feeding weeks or even months before spawning, obviously affects the digestive proteases. Torrissen and Torrissen (1984) observed the total activities of digestive proteases of maturing Atlantic salmon (Salmo salar) in June to be significantly higher than of immature fish.

This experiment was conducted to study the changes in proteases in the tissues of the digestive tract of Atlantic salmon during sexual maturation. The zymogram of the tryptic-like enzymes was also observed and the variants were designated. The level of carotenoids in the flesh, plasma and ovaries during this maturation period were simultaneously investigated.

MATERIALS AND METHODS

The eggs of Atlantic salmon (Salmo salar) were obtained from three different Norwegian fishfarms; A/S Bolaks, Eikelandsosen; Normann Misje, Misje; and Torris-laks, Halsa. The eggs were hatched at Matre Aquaculture Station in early 1979. After one year (summer 1980),the smolts were marked by fin cutting and freezed marking techniques and transferred to Austevoll Aquaculture Station, Storebø. There they were reared in sea pens and fed a moist pellet diet (Table 1).

The investigation was carried out in the third year in the sea, from March until December 1983. The temperature at 2 m depth during this period is shown in Fig 1. The fish were starved 24 hrs before sampling, and 14-15 fish were randomly sampled each month, about 2-10 immature fish, 2-5 maturing males and 2-8 maturing females.

The fish were killed, and a 3-4 ml blood sample was withdrawn from the heart into an evacuated blood collection tube containing 0.05 ml of 17% EDTA(K3) as anticoagulant.

About 1 g of the muscle, by a vertical cut in the abdominal cavity between the pelvic fin and anus, was sampled together with the digestive tract and the ovaries. The samples were frozen until analyses. Body weight and length were measured before sacrifice. Sex and status of maturation were recorded.

The blood samples were centrifuged at 3000 rpm for 10 min to separate the serum, and kept cool until analyses.

Protease assay

The digestive tract of individual fish was emptied, defatted and divided into 2 sections; the stomach, and the pyloric caeca including the intestine. Each section was used for the determination of tissue proteases. The methods for the extraction of proteases and the assay of proteolytic activities were performed according to Rungruangsak and Utne (1981),except that the homogenization was conducted using an Ultra-turrax homogenizer. The peptic-like activity of the stomach extract was assayed at 37.5°C while the tryptic-like activities of the pyloric caeca and intestinal extract at 52.5°C (Torrissen, 1984a) . The assay was done in triplicate. The total protease activities resulted from the sum of the activities of the stomach and pyloric caeca-intestinal extracts.

Electrophoretic zymogram of tryptic-like enzymes

The enzymes from the pyloric caeca-intestinal extract of individual fish were used for identifying the tryptic-like enzyme on isoelectrofocusing electrophoresis in agarose gel. The method was done according to Torrissen (1984a). In order to promote standardization in the nomenclature of enzyme variants, the system proposed by Allendorf and Utter (1979) is used in this experiment. The abbreviation for the tryptic-like enzyme is TRP. Since the samples were applied near the anode end,the hyphenated numerals designating multiple loci are listed in order of decreasing mobility towards the cathode.

Caroteniods assay

The carotenoid content was determined by the method described by Torrissen and Naevdal (1984) and identified by the method described by Lambertsen and Braekkan (1971).

Statistical analysis

The statistical analysis was carried out by using the BMDP statistical software (Dixon, 1981) and RS/1 (Bolt Beranek and Newman, Inc., Cambridge, Massachusetts).

RESULTS

Protease activities

The weight of immature fish varied between 1.4 to 11.6 kg, maturing males from 1.9 to 15 kg and maturing females from 1.9 to 10.5 kg. The total protease activities changed during sexual maturation (Fig. 2). At the early stage, in April, the enzyme activities were significantly higher ($P=0.05$) in both males and females than in immature fish. The activities were higher on average in maturing females (6.75+-4.36 µmol tyr/hr/mg protein) than maturing males (5.56+-1.91 µmol tyr/hr/mg protein) but this difference was not significant. The protease activities of maturing fish decreased to a level as immature fish in May and in June in maturing females. The total protease activities of maturing males decreased significantly in June ($P<0.05$) compared to immature fish. In maturing females, the enzyme activities decreased in July and were significantly lower than in immature

fish in August ($P<0.005$). In September, about 10-20% of the total enzyme activities remained in maturing fish of both sexes. The total protease activities were maintained at this low level until the fish were sexual mature in November and December. When the total protease activities were separated into peptic and tryptic-like activities (Fig.3), the response of the digestive proteases during sexual maturation was higher in tryptic-like enzymes than in peptic-like one, especially at the early stage of maturation. The change in the activity pattern of the total protease activities during this maturation period corresponded to the tryptic-like activities (Figs.2 and 3).

The results of March in Figs.2 and 3 were from the protease activities of the fish which had been mature in winter 1982. The enzyme activities of the postspawning fish had not yet returned to the normal level.

Electrophoretic zymogram of tryptic-like enzymes

According to the random sampling, 14 different families of Atlantic salmon were sampled, 11 groups from A/S Bolaks, 1 group from Normann Misje and 2 groups from Torris-laks.

Fig.4 represents the samples from the whole experimental period. Among 14 families from the three fishfarms, four different phenotypic variations in tryptic-like enzymes from pyloric caeca and intestine were observed on isoelectrofocusing electrophoresis in agarose gel. Three common isozymes designated TRP-1(100), TRP-2(100) and TRP-3, were detected in all groups (Fig.4a and b). Fig.4c and d showed one and three isozyme variations respectively, at higher pIs, TRP-1(86), TRP-1(69) and TRP-1(56). Another variation at lower pI, TRP-1(113), was

observed between TRP-1(100) and TRP-2(100) (Fig.4e). The TRP-2(100) was the strongest staining. The fish from eight families were found to have only three common isozymes as Fig.4a and b. They were distributed in all the three fishfarms. Two families from A/S Bolaks were found to have the same variation as Fig.4d and the other two families as Fig.4e. One family from A/S Bolaks and another one from Torris-laks were observed to have the same isozyme variation as shown in Fig. 4c. The TRP-1 and TRP-2 isozymes exhibited activities on the electrophoresis until the middle stage of sexual maturation in July. The TRP-3 seemed to decrease in its activity at the early stage of maturation,as early as in April (Fig.4e,f,g,h,i and j). At the late stage,in August,the activities of all isozymes started to decrease and one interlocus between TRP-2(100) and TRP-3,designated TRP-2(108) , was developed as shown in Fig.4k,l and m. At the latest stage before maturation (in October) and when the fish were mature (in November and December),the enzyme activities were too weak to be detected on the electrophoresis.

Carotenoids content

Astaxanthin was found as the dominant red carotenoid, but small amounts of cantaxanthin were also identified.

There was no significant difference in the astaxanthin level between males and females either in plasma (P=0.70), or in the flesh (P=0.17).

The carotenoid level in plasma during the experimental period for immature and maturing fish are shown in Fig. 5. The difference between immature and maturing fish, the change during the experimental period and the interaction between time and

maturation were all significant at $P=0.050$, $P<0.000$ and $P<0.000$ respectively. There was also a significant ($P<0.000$) covariance between the tryptic-like activities in the pyloric caeca and the intestine (Fig.6), but no significant ($P=0.54$) covariance with the peptic-like activity in the stomach.

Also the difference in flesh pigmentation between maturing and immature fish, the change during the experimental period and the interaction between maturation and time were significant ($P<0.000$). However, the difference in total amount of astaxanthin per fish was not significant ($P=0.15$).

The level of astaxanthin in maturing eggs decreased during the experimental period, while the level in immature eggs was constant. The difference between immature and maturing and the interaction between experimental time and maturation were significant, $P<0.000$ and $P=0.006$. The total amount of astaxanthin in maturing eggs increased during the experimental period, while the total amount in immature ovaries was constant. The difference was significant, $P<0.000$ (Fig 5).

DISCUSSION

It was possible to discriminate maturing and immature fishes by the development of the gonads as early as in April. The total amount of astaxanthin in the ovaries increased during the whole experimental period from April to November. However, the growth of the ovaries was relatively higher than the deposition of astaxanthin which made the level in the ovaries decrease during the period from June to September.

The mobilization of the flesh astaxanthin was detected by a reduction of the level of astaxanthin in maturing fish compared to immature from the end of June. The flesh of maturing fish in toto retained about 40% of the astaxanthin level in immature fish. This value is higher than the 1-5% found in sockeye salmon (Oncorhynchus nerka) by Crozier (1970). However, factors other than comparative differences might be the main effects. The level of astaxanthin in the flesh and also the availability of dietary astaxanthin is thought to contribute significantly to the rate of transfer of astaxanthin from flesh to ovaries.

The level of carotenoids in plasma are probably mainly influenced by the absorption of carotenoids from the diet. Fig. 5 shows a peak in the plasma carotenoid level for both immature and maturing fish during summertime, the period when water temperature (Fig. 1) and also the feed consumption was at the maximum level. Even though the digestibility of the main feed constituents influence the digestibility of the minor components, the absorption of carotenoids from the feed should be relatively independent of the proteolytic activity in the digestive tract. The significant covariance between the carotenoid levels in plasma and the tryptic like activity in the pyloric caeca and intestine are likely to be an effect of both the tryptic activity and the plasma level of carotenoids depending on the amount of feed consumed.

However, the significant difference between immature and maturing fish, and also the significant interaction between time and maturation, show that the process of maturation also contributes to the plasma level of carotenoids. The carotenoid level in the plasma of maturing fish dropped relatively more

than for the immature fish in July and August. This is probably because of the reduced food consumption. However, during September and October, the level was higher in maturing than immature, and this may be an effect of transportation of carotenoids from flesh to the ovaries and the skin.

At all stages the level of astaxanthin in the ovaries was higher than in the flesh, showing a higher affinity for carotenoid deposition in the ovaries. This, together with the mobilization of the muscle astaxanthin, and the transportation and deposition in the ovaries during the period of sexual maturation supports the hypothesis of a function of carotenoids in reproduction or early life. So far, no adequate scientific data have shown a specific function of carotenoids in reproduction (Tacon, 1981; Torrissen, 1984b) but Torrissen (1984b) found a growth promoting effect of astaxanthin or cantaxanthin supplementation in the startfeeding diet to Atlantic salmon fry.

The protease activity in the digestive tissue and the change in the electrophoretic zymograms during the period from April until December are clearly influenced by the sexual maturation process. Similar results were observed by Torrissen and Torrissen (1984), but the protease activity in sexually maturing fish were significantly higher than in immature in June. The shift in time compared to the present experiment is probably due to variation in the environmental condition from year to year.

A direct relation between digestive protease activities, the pattern on the electrophoretic zymograms and the sexual maturation is unlikely. The change in activities is probably a result of change in the feed consumption. The low activity in the post spawners in March, and the significant covariation

between astaxanthin in plasma and the tryptic-like activities support this. Based on this hypothesis, the tryptic-like activities are more directly influenced by the feeding behaviour than the peptic activity; or the peptic activity may have sinusoidal response to feeding compared to the relatively steady tryptic activities.

Apart from the effect of maturation and feed consumption, the variation of the migrating TRP-1 bands, TRP-1(113), TRP-1(86), TRP-1(69) and TRP-1(56), in the immature fish may reflect a genetic variation in Atlantic salmon.

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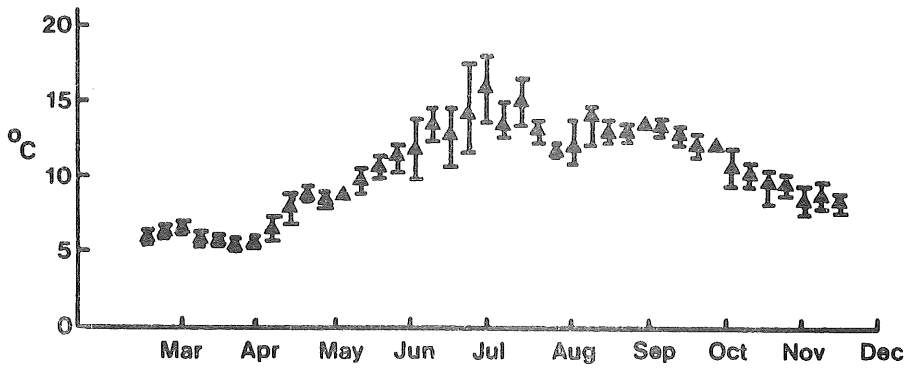

Fig.1. The temperature in seawater at Austevoll at 2m depth. The bars indicate the weekly range. The ticks on the time axis are at the 15th of each month.

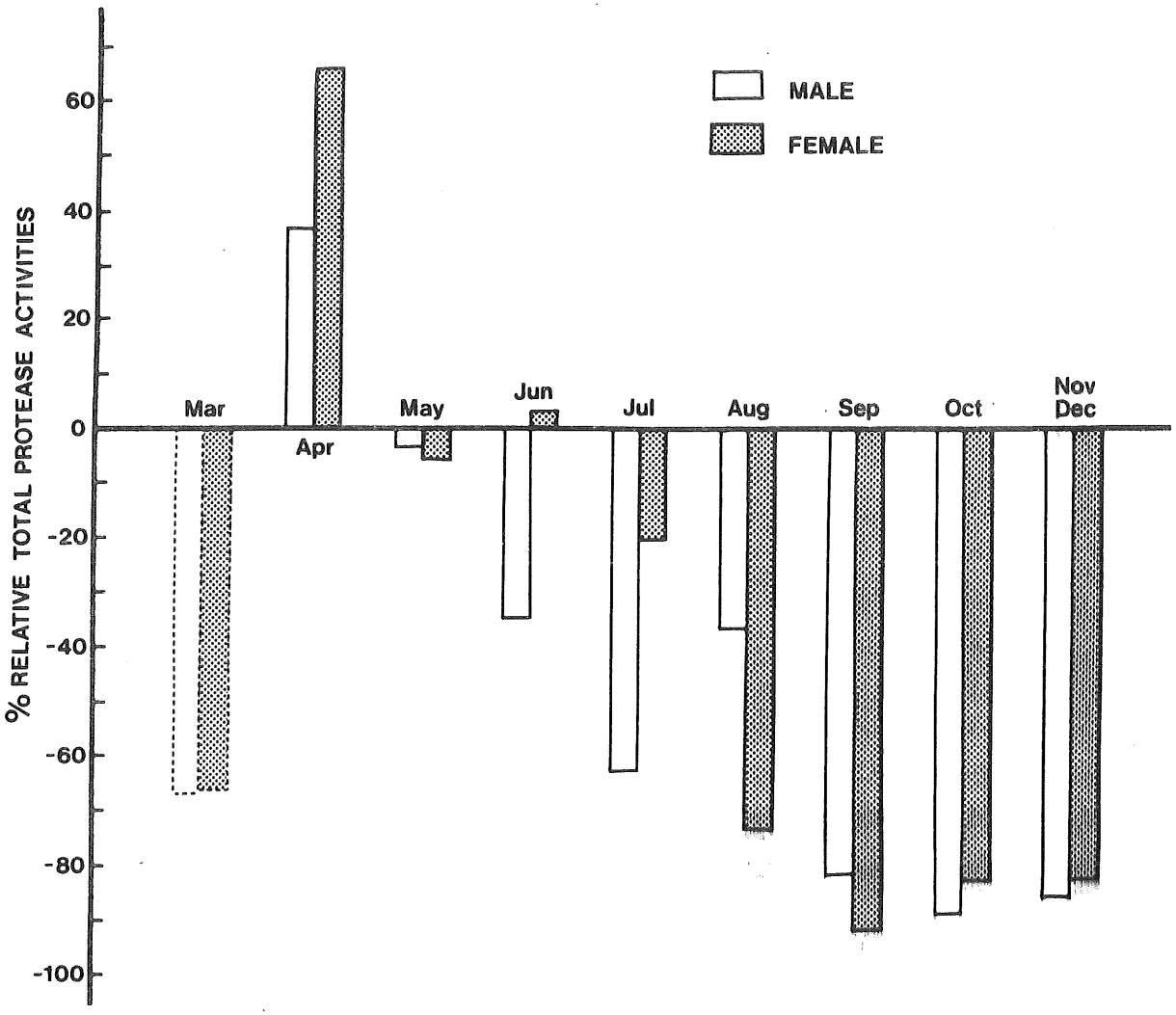

Fig.2. The percent relative total protease activities in the digestive tissues of maturing male and female Atlantic salmon compared to immature fish.

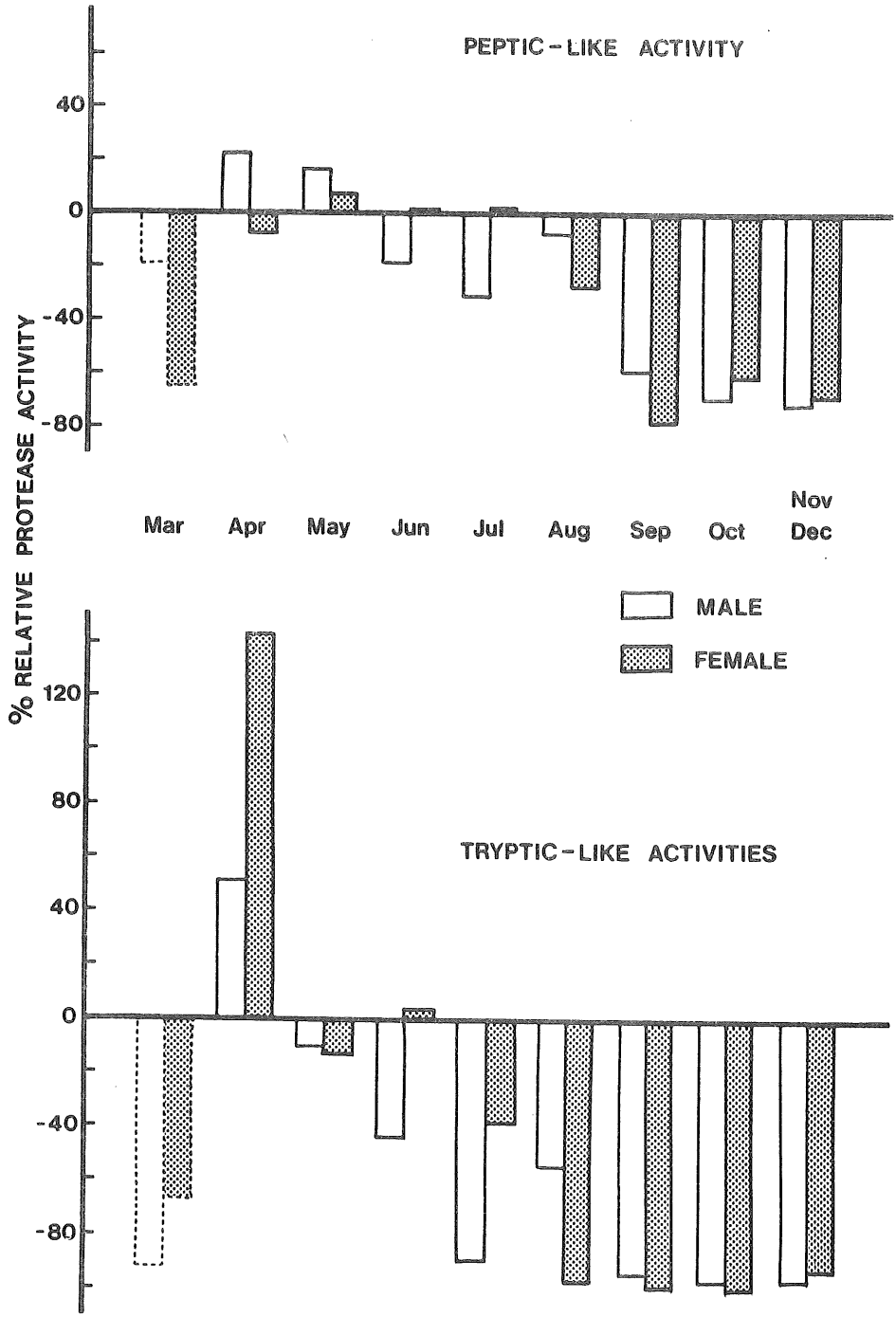

Fig.3. The percent relative activities of peptic-like and tryptic-like enzymes in the digestive tissues of maturing male and female Atlantic salmon compared to immature fish.

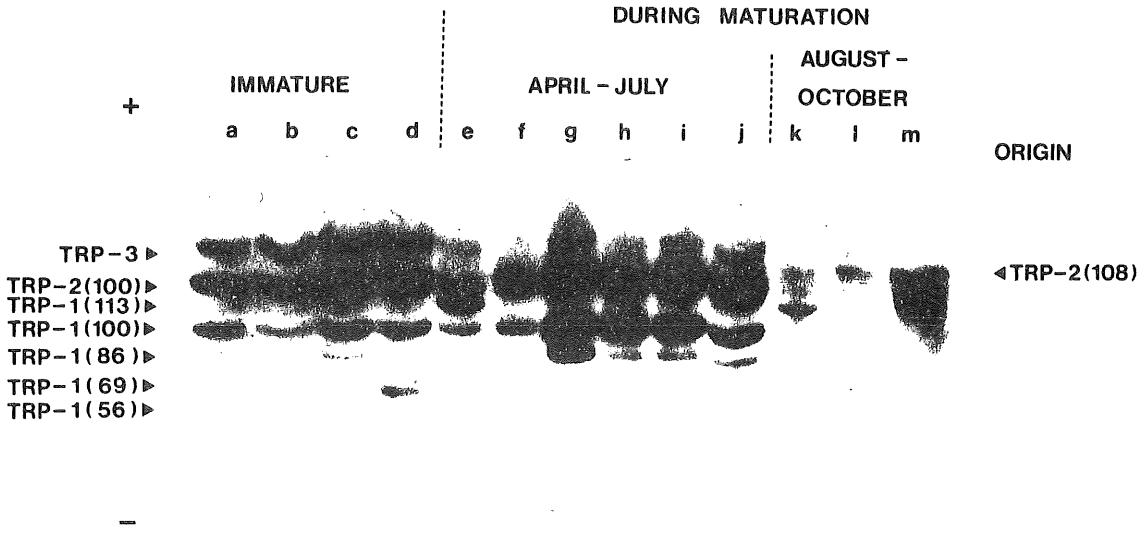

Fig.4. The electrophoretic zymograms of tryptic-like enzymes of immature and maturing Atlantic salmon during the maturation period.

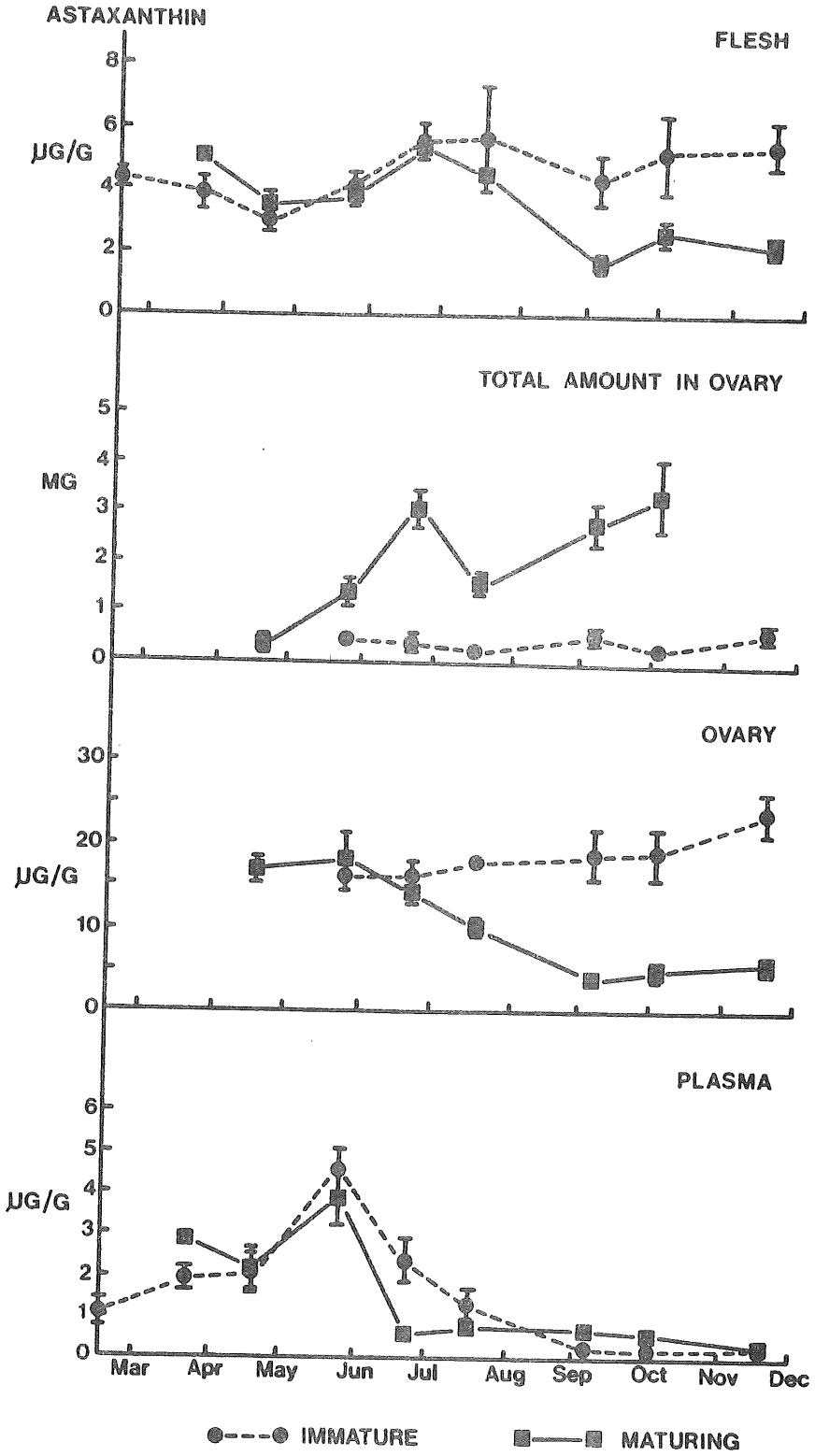

Fig.5. The levels of astaxanthin (±S.E.M.) in flesh, ovary and plasma, and the total amount of astaxanthin in the ovaries of immature and maturing Atlantic salmon. The ticks on the time axis are at the 15th of each month. (S.E.M. = Standard Error of Mean)

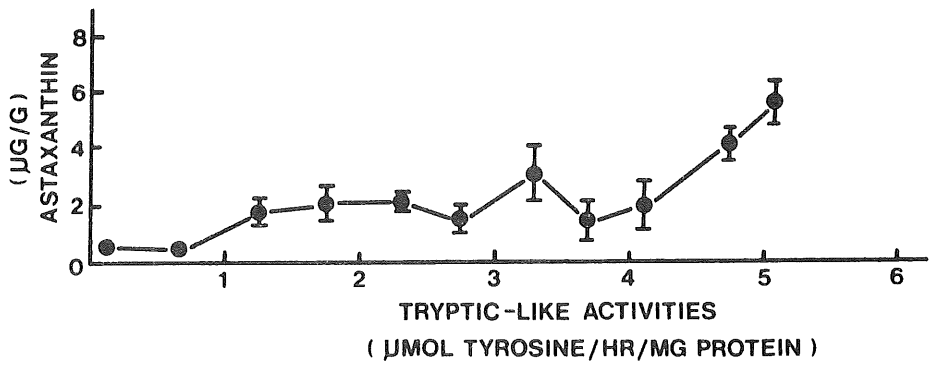

Fig.6. The relation between tryptic-like activities in the digestive tissues and the level of astaxanthin (±S.E.M.) in the plasma of Atlantic salmon during the period from March until December. Data from both immature and maturing fish were used. (S.E.M. = Standard Error of Mean)

Table 1. The composition of the moist pelleted feed.

Component	Weight (%)
Fishmeal (Norsea mink)	33.7
Capelin	33.7
Krill	13.5
Binder mixture*	11.2
Capelin oil	4.5
Vitamin mixture**	2.2
Vitamin E concentrate	1.1
Vitamin C	0.1

* According to Skretting (1981a).

** According to Skretting (1981b).