In: Salmon ISBN: 978-1-63117-570-1
Editors: Patrick T. K. Woo and Donald J. Noakes

Chapter 6

ATLANTIC SALMON, *SALMO SALAR* L.: GENETIC VARIATIONS IN PROTEIN METABOLISM AND GROWTH

Krisna Rungruangsak-Torrissen*
Institute of Marine Research, Ecosystem Processes Research Group,
Matre Research Station, Matredal, Norway

ABSTRACT

Atlantic salmon, *Salmo salar* L., is an anadromous species living in fresh water during the first year(s) of life until it smolts, and later in the sea from post-smolts until maturation before returning to fresh water to spawn. The early life period in fresh water and the first sea winter are critical periods when genetics, food qualities, and environmental conditions can affect growth rates. As a carnivore, dietary protein is a very important key nutrient for growth, and trypsin is the key protease that activates other pancreatic zymogens including chymotrypsinogen. This has made trypsin important for genetic studies on protein metabolism and the protease activity ratio of trypsin to chymotrypsin (T/C ratio) the important factor for digestive efficiency and growth.

Trypsin has different isoforms. Variations in genetic expression of trypsin isozyme patterns (trypsin phenotypes) of individual Atlantic salmon result in different abilities of the fish to digest the same protein and optimize food utilization and growth. Changes in trypsin phenotypes can be induced by temperature (at egg incubation and first feeding periods) and by dietary quality at the very early life stage. Changes in environmental conditions affect trypsin gene expressions at molecular and protein levels, regardless of genetic expression of parents. The effects can be maintenance ration, consumption, digestion, absorption and transport of free amino acids, insulin secretion, protein growth efficiency (ratio of protein to lipid – P/L ratio), health, maturation, and behaviour during the whole life cycle. Fish possessing different trypsin genotypes have different temperature preferences for optimizing food digestion for utilization and growth, which result in different growth rates and temperature distributions of the fish in natural ecosystems. Genetically manipulated fish, such as triploid Atlantic salmon, have less food utilization efficiency than their diploid counterparts. Molecular cloning and

* Corresponding Author address: *Matre Research Station, N-5984 Matredal, Norway*; Email: *Krisnart@imr.no.*

characterization of trypsin isozymes show very little genetic variation in trypsin genes. So far, trypsin clones and trypsin isozymes have never been matched, probably due to too few differences in sequences and the knowledge of gene expression is still limited. Studies on trypsin genotypes have provided new insights that can be exploited and integrated into other research fields to elucidate genetics of growth performance quality through food utilization under different environmental conditions.

Keywords: Trypsin isozymes, trypsin/chymotrypsin ratio, free amino acids, hydroxyproline, insulin, protein/lipid ratio, RNA/protein ratio, pyloric caeca, muscle

INTRODUCTION

Atlantic salmon, *Salmo salar* L., juveniles spend one to several years (depending on the environmental conditions) before they undergo smoltification and start their oceanic life stage. Their marine life stage may vary from a few months to four years depending on their genetics, water temperature, and feeding condition. At the onset of maturation (1–3 winters) they start their migration towards the coast where they head to their natal rivers to spawn. Many Atlantic salmon do not recover after spawning; about 25% of survivals return to the ocean before returning to spawn for a second time. Only a small fraction may live to spawn for a third time and males as repeated spawners of up to 150 cm and 40 kg have been recorded (Holm et al. 2004).

During the whole life cycle of Atlantic salmon, biochemical changes either due to internal factors (genetics, age, growth stage) or external factors (temperature, light, vaccine, feeding condition) will affect their growth. The aim of this chapter is to review studies on the effects of genetic differences in trypsin phenotypes on growth in association with food utilization efficiency, especially in protein metabolism, in Atlantic salmon and other aquatic animals in aquaculture as well as in natural ecosystems.

GENETIC STUDIES OF TRYSIN GENOTYPES IN ATLANTIC SALMON

Trypsin Genotypes in Association with Fish Size and Trypsin Activity

The study on genetic variations of trypsin phenotypes in Atlantic salmon in association with fry sizes was first performed by Rungruangsak-Torrissen (Torrissen 1987). There are different trypsin phenotypes (Figure 1) and the technique for identifying trypsin isozyme patterns (trypsin phenotypes) is by using isoelectric focusing (IEF) on Agarose IEF gel at pH 4–6.5 (Torrissen 1984), using *N*-benzoyl-*L*-arginine-*p*-nitroanilide (BAPNA) as the substrate in the presence of sodium nitrite and naphthylethylenediamine for diazotizing and coupling with produced nitroaniline to develop a bright purple colour when the gel is dipped into trichloroacetic acid (Dahlmann and Jany 1975). At a rearing temperature of around 8 ^{o}C, the fry designated *TRP-2*92/92* genotype (Figure 1) showed average weights (pattern numbers 4–6: 15.21±0.70 g; pattern numbers 19–20: 14.10±1.33 g) significantly higher than those of other genotypes (7.41–10.13 g) (Torrissen 1987). There were clear associations between fish size and trypsin genotypes, regardless of families.

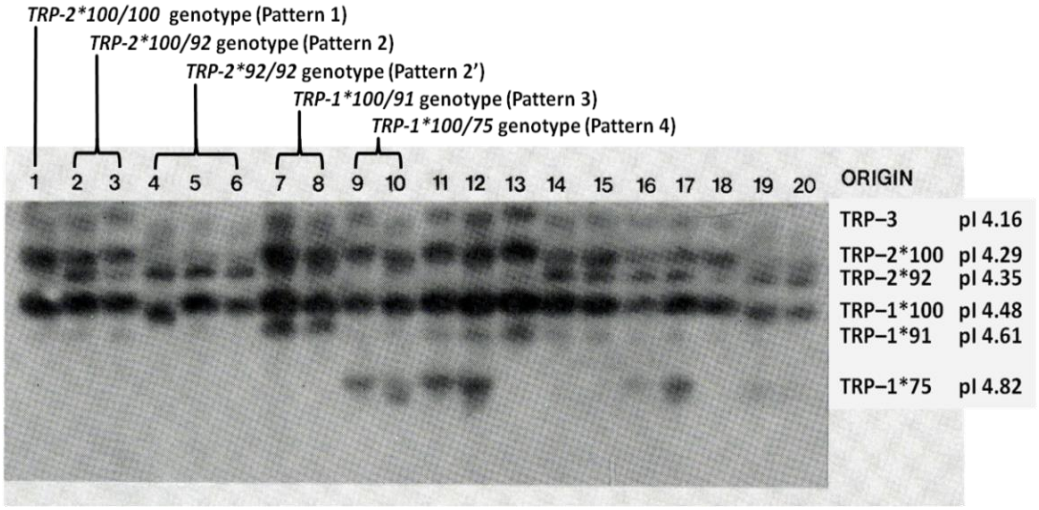

Figure 1. The first electrophoretic zymograms (on Agarose IEF gel pH 4–6.5) of different trypsin isozyme patterns (trypsin phenotypes) of Atlantic salmon fry. (Adapted from Torrissen [1987], with permission from Elsevier B.V.). The designated names of trypsin isozymes were adapted from Torrissen et al. [1993], and the isoelectric points (pI) were from Rungruangsak-Torrissen et al. [1998]. The trypsin genotypes of the *TRP-1* and *TRP-2* systems and designated pattern names are illustrated on top of the picture. The rests are genotypes of the combinations of different trypsin isozyme patterns.

In contrast to the weight, the specific activity of trypsin (previously named trypsin-like, and now trypsin, see Rungruangsak Torrissen and Male 2000) from salmon groups with *TRP-2*92/92* genotype (pattern numbers 4–6: 3.59±0.29; pattern numbers 19–20: 3.13±0.68 µmol tyrosine h^{-1} mg $protein^{-1}$) was significantly lower than the groups without the *TRP-2*92/92* genotype (5.31–12.42 µmol tyrosine h^{-1} mg $protein^{-1}$). The total trypsin activity of the *TRP-2*92/92* genotype (pattern numbers 4–6: 16.26±1.41; pattern numbers 19–20: 10.02±1.47 µmol tyrosine h^{-1} fry^{-1}) was similar to the other fish groups (11.59–18.45 µmol tyrosine h^{-1} fry^{-1}), regardless of families (Figure 2). However, when the weight was added as a covariant, the adjusted values of the total trypsin activity from the groups with *TRP-2*92/92* genotype were also significantly lower than those of the other genotypes (Torrissen 1987). The controversial result between trypsin specific activity (observed in pyloric caecal tissue) and the weights of different trypsin genotypes could be explained by more recent studies, which indicated a higher secretion of trypsin into the pyloric caecal lumen by *TRP-2*92* salmon compared to those without the variant (Rungruangsak Torrissen and Male 2000). Therefore, trypsin activity remained lower in the pyloric caecal tissue of the *TRP-2*92* salmon (Figure 2) because the total trypsin activity per fry (Figure 2) as well as in sum of pyloric caecal tissues and lumen (Rungruangsak Torrissen and Male 2000) were similar between the fish with different trypsin genotypes. The results were also in line with the observations by Male et al. [1995] that trypsin genes seemed to be stable, and although the pattern of expressed genes varied extensively, the expression of trypsinogen mRNA was quantitatively similar between individual salmon.

Moreover, trypsin activity in the pyloric caeca with food content is not necessarily higher in faster growing fish. It depends on how well the fish adapt to new food and new environment, as shown in a later section that food utilization efficiency and growth are related to the activity

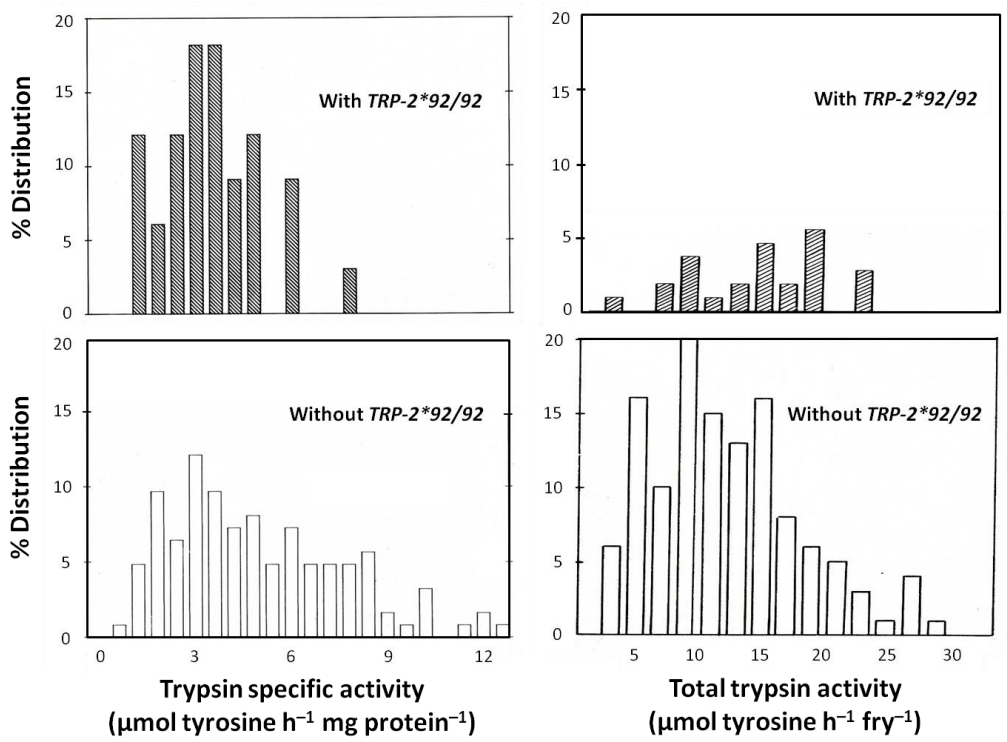

Figure 2. The distributions of trypsin specific activity and total trypsin activity of Atlantic salmon fry with and without TRP-2*92/92 genotype. (Adapted from Torrissen [1987], with permission from Elsevier B.V.). The enzyme activity was previously named trypsin-like and now trypsin (Rungruangsak Torrissen and Male 2000).

ratio of trypsin to chymotrypsin (T/C ratio) independent of the specific activity levels of trypsin and chymotrypsin (Sunde et al. 2001; Blier et al. 2002; Rungruangsak-Torrissen et al. 2009a, 2009b; Rungruangsak-Torrissen 2012).

There were significant differences among families in weight and the total trypsin activity, and the covariance between weight and the total trypsin activity was significant. Within the same family, the individuals possessing the trypsin variant TRP-2*92 either heterozygote *TRP-2*100/92* (patterns numbers 2–3 and 14–17; Figure 1) or homozygote *TRP-2*92/92* (pattern numbers 4–6 and 19–20; Figure 1) were usually bigger than the others, and the families with higher percentage of individuals possessing the TRP-2*92 variant had higher average weights. Moreover, the increase in the relative intensity of the *TRP-2*92* allele corresponded with an increase in the mean weight of the fish (Torrissen 1987).

Later studies (Rungruangsak-Torrissen et al. 1998) demonstrated that each trypsin phenotype has a temperature preference for feed utilization and growth. The common trypsin isozyme TRP-2*100 was important at water temperature > 8 ^{o}C, while it was important for the expression of the trypsin variant TRP-2*92 at water temperature ≤ 8 ^{o}C, especially below 6 ^{o}C. The trypsin variant TRP-1*91 performed effectively at a wider temperature range than the variant TRP-2*92, but not at temperature ≤ 6 ^{o}C. The isozyme TRP-2*92 was the major variant (47%) in Norwegian salmon, while the variant TRP-1*91 was dominant (42%) in Scottish salmon. The Scottish salmon should be expected to live in the water with temperature

somewhat higher than the water where the Norwegian salmon live. The diversity of both common and variant trypsin isozymes is important for feed utilization efficiency and growth at varying temperatures. Genetic variation in trypsin isozyme patterns has been shown to be a primary factor affecting feed conversion efficiency and growth in Atlantic salmon under different rearing temperatures (Rungruangsak-Torrissen et al. 1998).

Spatial temperature distribution in the Norwegian Sea of the different trypsin isozyme characteristics of wild Atlantic salmon post-smolts confirmed that post-smolts with the genotypes *TRP-2*100/100*, *TRP-2*100/92*, and *TRP-2*92/92* were distributed at the estimated ambient temperatures of 9.3 ^{o}C, 8.7 ^{o}C, and 7.7 ^{o}C, with the weights of 132.6±12.2 g, 136.5±14.9 g, and 234.3±24.9 g, respectively. Fish possessing *TRP-2*100/92* genotype were significantly larger in the areas above 68^{o}N close to the 8 ^{o}C isotherm (188.0±18.9 g (n=6)), compared to the rest of the same trypsin genotype (102.2±11.4 g (n=9)) caught at the isotherms of > 8 ^{o}C in the Norwegian Sea. However, off the Hebrides area where the estimated ambient temperature was 10.2 ^{o}C with relatively much higher ichthyoplankton densities (200–2000 at 0–50 m depth) than the Norwegian Sea (5–60 at 0–50 m depth, probably due to higher grazing), the post-smolts were forced to stay in this area for feeding, and because of the high ambient temperature the weights of the fish for the genotypes *TRP-2*100/100*, *TRP-2*100/92*, and *TRP-2*92/92* were 63.9±7.1 g, 62.4±3.2 g, and 57.9±4.8 g, respectively. Although the fish caught in the Norwegian Sea were on average larger, they tended to be younger than those off the Hebrides area (Rungruangsak-Torrissen and Stensholt 2001; Rungruangsak-Torrissen 2012).

When the temperature was high and with food abundant (off the Hebrides area), the advantages of the salmon with *TRP-2*92/92* genotype for food utilization and growth were reduced. On the other hand, when foods were limited and the thermoclines were clear in the Norwegian Sea, the *TRP-2*92/92* as well as the *TRP-2*100/92* genotype were distributed in lower temperature areas. Trypsin isozyme patterns were identifiable in more than 80% of the post-smolts caught and they possessed these three trypsin genotypes. The variants TRP-1*91 and TRP-1*75 were not observed in any of the post-smolts and only one adult salmon of 2,645 g possessing the variant TRP-1*91 was caught off the Herbrides area. Atlantic salmon populations do not seem to disperse randomly in the sea. In the Norwegian Sea, food organisms dominating in the stomach of the post-smolts were crustaceans (*Parathemisto* spp.), krill, herring and redfish larvae, whereas off the Herbrides area blue whiting larvae were the only food item identified.

Abundance, type, and size of foods may force fish to stay in the area with high occurrence of food organisms, and this can reduce the advantages of optimizing food utilization and growth in certain genotypes due to un-suitable ambient temperature. During sea migration and if the availability of suitable food is reflected by the ichthyoplankton index, growth of Atlantic salmon post-smolts will be affected by food utilization efficiency at different ambient temperatures and dependent on the trypsin genotypes of the individuals (Rungruangsak-Torrissen and Stensholt 2001; Rungruangsak-Torrissen 2012).

Further studies on trypsin genotypes have led to a better understanding on the associations of different biological processes in living organisms, and confirmed the temperature preferences of the different trypsin genotypes for optimizing food utilization (Rungruangsak-Torrissen et al. 2006). Atlantic salmon parr possessing the different trypsin genotypes of *TRP-2*100/100*, *TRP-2*100/92*, and *TRP-2*92/92*, were reared under different temperatures (Figure 3). During routine feeding, the slopes of the regressions between trypsin

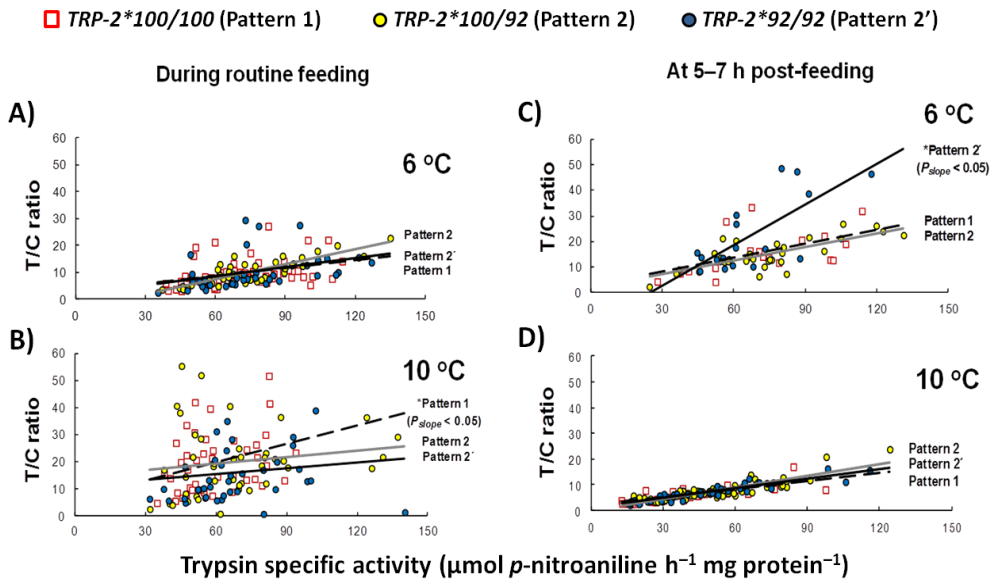

Figure 3. Effects of water temperatures (6 °C and 10 °C) on the relationship between trypsin specific activity and the activity ratio of trypsin to chymotrypsin (T/C ratio) in the pyloric caeca with food content from Atlantic salmon parr with different trypsin genotypes of *TRP-2*100/100*, *TRP-2*100/92*, and *TRP-2*92/92* (see Figure 1). The samples were collected (A and B) during routine feeding, and (C and D) at 5–7 h post-feeding. (Adapted data from Rungruangsak-Torrissen et al. [2006], with permission from Springer Corp.).

specific activities and the activity ratio of trypsin to chymotrypsin (T/C ratio) values, which correlate with growth rate, were similar among the three trypsin genotypes at 6 °C (Figure 3A), while the slope was higher in the *TRP-2*100/100* genotype than the *TRP-2*100/92* and *TRP-2*92/92* genotypes at 10 °C (Figure 3B). However, the slope of the post-prandial regressions between trypsin specific activities and the T/C ratio values was higher in the *TRP-2*92/92* genotype at 6 °C, compared to the *TRP-2*100/100* and *TRP-2*100/92* genotypes (Figure 3C), while the post-prandial regressions were similar among the three genotypes at 10 °C (Figure 3D). This was probably due to the amount of feed required for growth at 6 °C was less than at 10 °C, and a 30 min re-feeding probably was sufficient for the requirement at 6 °C but not at 10 °C. However, the results indicate that the genotype feeding at suitable temperature will have a relatively high diet utilization resulted from high levels of trypsin specific activity and the T/C ratio.

Differences in hatching and start-feeding temperatures and in trypsin genotypes could affect growth and the expressions of trypsin and chymotrypsin and the T/C ratio values in salmon parr (Table 1). Trypsin specific activity is affected by the interaction between start-feeding temperature and trypsin genotype. The T/C ratio is affected by start-feeding temperature, while chymotrypsin specific activity is influenced by both hatching and start-feeding temperatures (Table 1). Variations in trypsin genotypes will influence fish growth at different temperatures through variations in food utilization, which is influenced by differences in the enzyme expressions of trypsin and chymotrypsin in the pyloric caeca.

Trypsin isozymes could be differentiated in the narrow pH range of 4–6.5 (Figure 1) and cationic isoform(s) of trypsin could exist at pH > 10 (Rungruangsak Torrissen and Male 2000).

Table 1. Three-way ANOVA of the effects of temperatures (at hatching and start-feeding) and trypsin genotypes on weight and the expressions of trypsin and chymotrypsin (expressed as μmol *p*-nitroaniline h^{-1} mg $protein^{-1}$) and the protease activity ratio of trypsin to chymotrypsin (T/C ratio) during the first winter in Atlantic salmon parr, with significant effects shown by the bold *P* values of < 0.05. (Data from Rungruangsak-Torrissen et al. [2006], with permission from Springer Corp.).

General effects	df	Probability values			
		Weight (g)	Trypsin	Chymotrypsin	T/C ratio
Hatching temperature (HT)	1	**0.0000**	0.6326	**0.0132**	0.0825
Start-feeding temperature (SFT)	1	**0.0000**	0.1055	**0.0000**	**0.0000**
Trypsin genotypes (TRP)	2	**0.0123**	0.7936	0.3278	0.4784
HT × SFT	1	**0.0000**	0.1918	0.7508	0.1745
HT × TRP	2	**0.0075**	0.3477	0.0594	0.2843
SFT × TRP	2	0.0755	**0.0256**	0.6388	0.0690
HT × SFT × TRP	2	**0.0216**	0.7903	0.2469	0.2941
Error	246				

Trypsin variants TRP-1*91 and TRP-1*75 were about 13.5% and 17.4%, respectively, in Norwegian salmon aquaculture (Torrissen 1987). A decade later, the trypsin variant TRP-1*91 represented only 10% in Norwegian salmon aquaculture without any detection of the variant TRP-1*75 (Rungruangsak-Torrissen et al. 1998). The isozyme TRP-2*92 was the major variant found in salmon aquaculture (Torrissen 1987; Rungruangsak-Torrissen et al. 1998) as well as in the natural marine ecosystem of the North Atlantic Ocean (Rungruangsak-Torrissen and Stensholt 2001). When the rearing temperature increased to more than 10 ^{o}C to promote growth rate in salmon aquaculture, the expression of the trypsin variant TRP-2*92 was disturbed (unpublished result). If the variant TRP-2*92 disappears, it will affect the survival and growth of the post-smolts in marine ecosystems where the temperatures are usually low. Increased rearing temperatures may be beneficial for aquaculture for food production, but not for sea ranching and conservation.

Cloning and characterization of trypsin isozymes in Atlantic salmon have been performed, and five clones containing near full-length transcripts (four encoded anionic forms and one encoded cationic variant) have been revealed (Male et al. 1995). So far, trypsin clones and trypsin isozymes have never been matched, probably due to too few differences in sequences and the knowledge of gene expression is still limited. So far, molecular technique has only been used for genetic structure and species identification. However, real functional genomics studies aimed at knowing the proteins and their functions are very important and more practical for understanding the biological significance in living organisms. The study of trypsin isozyme expressions in Atlantic salmon by Rungruangsak-Torrissen and her research team is a unique example, and has been the most intensive investigation providing significant insight of basic knowledge on food utilization efficiency and growth performance quality in both aquaculture and natural ecosystems. The knowledge could be applied for other species. Such studies have never been performed elsewhere.

Development of Trypsin Isozyme Patterns and Heredity

Trypsin isozymes of Atlantic salmon alevins seemed to develop just after the first feeding, and in the families possessing *TRP-2*100/92* genotype, the trypsin variant TRP-2*92 seemed to develop later than the common isozyme TRP-2*100 (Torrissen 1987; Rungruangsak Torrissen and Male 2000). The same trypsin isozyme patterns were observed through the whole life cycle of Atlantic salmon; for example, early development (Rungruangsak-Torrissen et al. 1998), before smoltification (Figure 1), immature fish (Figure 4), and before and during sexual maturation (Figure 4). During the maturing process, the visual intensities of the trypsin isozyme TRP-3 seemed to decrease earlier than the other trypsin isozymes (April–July in Figure 4). At the late maturing stage, when food consumption was very low, the activities of all trypsin isozymes decreased as only weak intensities were detected, and a new trypsin isozyme band was detected between isozymes TRP-3 and TRP-2*100 (August–October in Figure 4). When the salmon matured and stopped feeding, the enzyme activities were too weak to be detected by the IEF electrophoresis (Torrissen and Torrissen 1985).

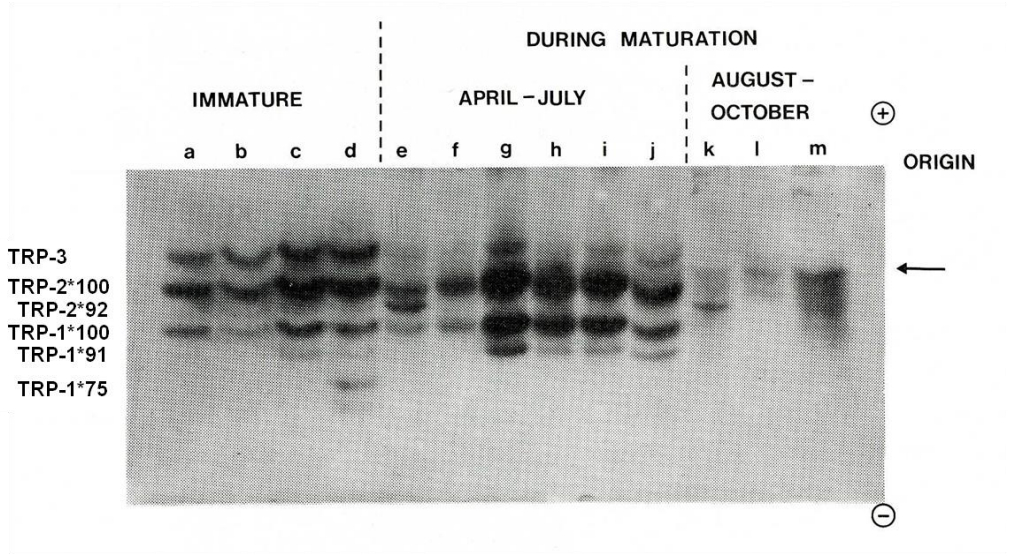

Figure 4. The electrophoretic zymograms (on Agarose IEF gel pH 4–6.5) of trypsin isozyme patterns of immature and maturing Atlantic salmon during maturing processes. The arrow indicates a new trypsin isozyme band detected at the late maturing stage. (From Torrissen and Torrissen [1985], with permission from Elsevier B.V.).

The appearance of the trypsin isozyme band detected at the late stage of maturation (shown by arrow in Figure 4) should not be due to the effect of low feed consumption as it was not detected in immature fish in November–December when the fish stopped active feeding. It may be a modification of trypsin isozyme during maturation or represent an isozyme that can be detected only when the activity of the TRP-2*100 is low (Torrissen and Torrissen 1985).

Heredity study of the polymorphic trypsin locus *TRP-1* and locus *TRP-2* by crossing individual fish with known trypsin genotype resulted in offspring with trypsin isozyme

patterns that could not be easily explained by ordinary disomic (Mendelian) inheritance (Torrissen et al. 1993). This might be due to the consequence of a tetraploid event in an ancestral salmonids or the underestimation of the number of heterozygotes if the isozyme alleles existed as a 3:1 ratio. Moreover, differences in hatching and first feeding temperatures could contribute to variations in the expressions of different trypsin isozymes in the offspring, regardless of genetic expression of parents, as changes in frequency distribution of trypsin isozyme patterns (Rungruangsak-Torrissen et al. 1998; Rungruangsak Torrissen and Male 2000) and protease activity ratio of trypsin to chymotrypsin (T/C ratio) (Rungruangsak-Torrissen 2002) were observed between fish hatched and start-fed at different temperatures (Table 2). The effects of warm hatching temperature (10 ^{o}C) on the expression of the common isozyme TRP-2*100, and of cold hatching temperature (6 ^{o}C) on the expression of the variant TRP-2*92 were observed. Surprisingly, the warm temperature (12 ^{o}C) at first feeding period increased the expression of the variant TRP-2*92 by promoting the occurrence of trypsin genotypes *TRP-2*100/92* and *TRP-2*92/92*. Late expression of the trypsin variant TRP-2*92 compared to the common isozyme TRP-2*100 during the first three weeks of first feeding period at 8 ^{o}C (Torrissen 1987; Rungruangsak Torrissen and Male 2000), and the increased occurrence of the variant when the alevins were start-fed at 12 ^{o}C (Rungruangsak-Torrissen et al. 1998, Rungruangsak Torrissen and Male 2000), demonstrated that the expression of the variant TRP-2*92 was able to be induced during the first feeding at 8–12 ^{o}C although it was manifested at temperature $\leq$ 8 ^{o}C, especially below 6 ^{o}C (Rungruangsak-Torrissen et al. 1998, Rungruangsak Torrissen and Male 2000). Changes in rearing temperature at later stages did not change the trypsin isozyme patterns.

Studies of protease specific activities of trypsin (T) and chymotrypsin (C) and the activity ratio of these two enzymes (T/C ratio) in these fish groups during the first winter (January) indicated that these enzyme values varied according to trypsin phenotypes, past temperature experience during early feeding, and present environmental temperature (Table 2). The salmon (*TRP-2*92/92*) with the trypsin variant effectively functioning at temperature < 6 ^{o}C showed relatively higher values of either trypsin specific activity or both trypsin specific activity and T/C ratio than the *TRP-2*100/100* salmon without the variant, if both genotypes had cold start-feeding temperature experience. These enzyme values were vice versa (pattern 2´ < pattern 1) if the fish had warm start-feeding temperature experience, regardless of hatching temperature. There was no difference in weight during the first winter between salmon parr of different trypsin genotypes within the same temperature control group, except for the group of warm hatching and warm start-feeding temperatures that the *TRP-2*100/100* salmon lacking the cold temperature variant were significantly smaller than the *TRP-2*100/92* and *TRP-2*92/92* salmon carrying the variant. Although the *TRP-2*100/100* salmon having warm start-feeding temperature experience seemed to be smaller than the *TRP-2*92/92* salmon during the first winter due to lacking cold temperature functioning isozyme TRP-2*92, they would grow faster later when water temperature increased as they had somewhat higher preceding trypsin specific activity and T/C ratio according to Rungruangsak-Torrissen et al. [2006].

Winter temperature had a higher adverse effect on *TRP-2*100/100* salmon if they had no earlier cold temperature experience. The *TRP-2*100/92* salmon showed better performance than the other genotypes at varying temperature control conditions (Table 2). Interestingly, trypsin specific activity and T/C ratio were higher, while chymotrypsin specific activity was lower, in higher growth salmon having warm start-feeding temperature experience than slower growth fish having cold start-feeding temperature experience, regardless of hatching temperatures and

trypsin genotypes. Trypsin is the key protease under condition favouring growth, while chymotrypsin plays a major role when growth opportunity is interrupted or limited (Rungruangsak-Torrissen et al. 2006).

Table 2. Number of sampled fish, frequency distribution, weight, specific activities of trypsin and chymotrypsin (μmol *p*-nitroaniline h^{-1} mg $protein^{-1}$) and activity ratio of trypsin to chymotrypsin (T/C ratio) of each trypsin genotype of Norwegian Atlantic salmon parr, hatched and start-fed at different temperatures. The values with different superscripts or with asterisk (*) are significantly different ($P < 0.05$). For the pattern ratios, within the same column, the ratios with different superscripts are significantly different ($P < 0.01$). (From Rungruangsak-Torrissen [2002], based on Rungruangsak-Torrissen et al. [1998] and Rungruangsak-Torrissen and Sundby [2000])

Temperature (°C)	Parameters (mean±sem)	Trypsin isozyme pattern and genotype				Pattern ratio	
		1 *TRP-2*100/100*	2 *TRP-2*100/92*	2' *TRP-2*92/92*	Others	1 : 2+2'	2 : 2'
Hatching 5.9±1.9 Start-feeding 5.6±1.3	Frequency	74 (0.27)	98 (0.36)	81 (0.30)	19 (0.07)	0.27 : 0.66[a]	0.56 : 0.44[a]
	Weight (g)	14.6±0.5	15.6±0.4	15.4±0.5			
	Trypsin	68.5±3.7	72.4±4.2	80.1±5.7			
	Chymotrypsin	7.9±0.6[a]	7.5±0.5[a]	9.6±0.7[b]			
	T/C ratio	9.9±1.0	10.7±0.9	9.3±1.1			
Hatching 5.9±1.9 Start-feeding 12.2±0.5	Frequency	40 (0.17)	101 (0.43)	84 (0.36)	10 (0.04)	0.17 : 0.79[b]	0.54 : 0.46[a]
	Weight (g)	34.6±2.0	33.0±1.0	34.8±1.4			
	Trypsin	84.1±12.1	77.6±3.4	72.5±3.7			
	Chymotrypsin	3.4±0.3	4.1±0.2	4.4±0.2			
	T/C ratio	26.2±4.8*	20.2±1.4	17.4±1.3*			
Hatching 9.6±1.2 Start-feeding 5.6±1.3	Frequency	81 (0.32)	111 (0.44)	50 (0.20)	11 (0.04)	0.32 : 0.64[a]	0.70 : 0.30[b]
	Weight (g)	17.5±0.7	20.0±0.9	16.1±1.0			
	Trypsin	72.3±3.6	71.3±4.6	73.4±5.3			
	Chymotrypsin	9.1±0.7	9.2±0.4	8.7±1.0			
	T/C ratio	9.5±0.9	8.5±0.9	11.7±2.1			
Hatching 9.6±1.2 Start-feeding 12.2±0.5	Frequency	49 (0.17)	146 (0.52)	69 (0.25)	17 (0.06)	0.17 : 0.77[b]	0.68 : 0.32[b]
	Weight (g)	46.3±4.5[a]	65.7±3.7[b]	61.2±4.8[b]			
	Trypsin	81.9±5.7	88.4±5.3	70.9±4.5			
	Chymotrypsin	4.9±0.4	4.9±0.2	4.9±0.3			
	T/C ratio	17.8±1.7	18.9±1.5	15.8±1.7			

Moreover, during early development, Atlantic salmon fry from families with higher frequencies of trypsin variant TRP-2*92 (both *TRP-2*100/92* and *TRP-2*92/92* genotypes) showed significantly higher increases in trypsin specific activity than the families without the variant during 4 months of the first-feeding period at 12 °C, while there were no differences in trypsin specific activity at 6 °C (Rungruangsak Torrissen and Male 2000). The presence of trypsin isozyme TRP-2*92 (*TRP-2*100/92* and *TRP-2*92/92* genotypes), was associated with increased growth rates manifested during the first few months after the first feeding in fresh water (Torrissen et al. 1993; Rungruangsak Torrissen and Male 2000), and during winter of the first sea-year (Torrissen 1991; Rungruangsak Torrissen and Male 2000). Studies in

Atlantic salmon parr from three different strains (Torrissen et al. 1993; Rungruangsak Torrissen and Male 2000) also indicated that the strain with higher frequency of trypsin variant TRP-2*92 was significantly larger and the differences in growth could be seen in either freshwater phase or seawater phase or both which depended on growth characteristics of each strain. No differences in weight were observed among the salmon lacking the variant from the different strains. The study suggests the association between higher growth rate and higher frequency of the salmon with *TRP-2*92* genotype (see Table 1 in Chapter 7).

Trypsin isozyme TRP-2*92 was shown to associate with increased growth in Atlantic salmon during the first feeding period (Torrissen 1987), from first feeding until post-smolts (Torrissen et al. 1993), and from smolts until maturation (Torrissen 1991). Larger fry carrying trypsin TRP-2*92 were not necessarily produced from larger eggs, and egg qualities (egg size, eyed egg period, mortality, hatching time) were not affected by differences in trypsin isozyme patterns of brooders (Torrissen et al. 1993).

Individual characteristics of trypsin isozyme pattern is developed and established in offspring during the egg incubation and the first feeding periods, whence phenotypic as well as genotypic changes can occur depending on the first environmental condition, regardless of genetic expression of parents. The pattern does not seem to change later in the life cycle. The early environmental experiences of the offspring will influence the development of well adapted trypsin isozyme patterns in the digestive system, which will affect food utilization efficiency, growth, and survival throughout the whole life cycle.

Trypsin Genotypes, Food Utilization and Growth

Genetic variations in trypsin isozyme expressions affected growth rates and trypsin specific activity levels in the pyloric caeca and intestine in Atlantic salmon at different temperatures. This led to investigations on whether these variations could be associated with differences in digestion and utilization of dietary protein. Torrissen and Shearer [1992] studied protein digestibility and feed conversion efficiency in Atlantic salmon with different trypsin genotypes at different life stages and reared at different temperatures and salinities. No differences in the apparent digestibility coefficient (ADC) of protein were observed between the salmon with and without trypsin variant TRP-2*92 in any cases (Table 3). Although the growth rates were not significantly different between the genotypes from smolts in fresh water and from post-smolts in water salinity 27, the relatively higher growth rates observed in salmon smolts and post-smolts with the variant, compared to those lacking the variant, did not associate with the ADC of protein (Table 3). During smoltification in water salinity of 16, the smolts with the variant had a significantly higher growth rate with higher feed conversion efficiency (FCE) and protein efficiency ratio (PER), but the ADC of protein was unfortunately not studied in these fish (Table 3).

Variations in water temperature, salinity, and fish size had no apparent effect on the ADC of protein. Also, two strains of Arctic charr, *Salvelinus alpinus* L., with different trypsin genotypes and growth rates indicated that the variations did not affect their ADC of protein (Torrissen and Barnung 1991). However, Torrissen et al. [1994, 1995] and Bassompierre et al. [1998] later differentiated the digestive ability among the different trypsin genotypes, which indicate that the ADC method is not sensitive enough for differentiating genetic differences in diet utilization.

Table 3. Apparent digestibility coefficient (ADC) of protein, specific growth rate (SGR), feed conversion efficiency (FCE: wet weight gain per dry feed consumed), and protein efficiency ratio (PER: wet weight gain per crude protein consumed), in Atlantic salmon with and without the trypsin variant TRP-2*92. The fish were from different life stages and reared at different temperatures and salinities. The values with asterisk (*) are significantly different between the two genotypes ($P < 0.03$). (Adapted from Torrissen and Shearer [1992], with permission from John Wiley and Sons, Inc.)

Parameters	Smolts (50 g) at 6 °C, salinity 0		Smolts (50 g) at 6 °C, salinity 16		Post-smolts (200 g) at 10 °C, salinity 27	
	With	Without	With	Without	With	Without
ADC of protein (%)	81.8	82.7	–	–	81.1±0.6	81.5±0.6
SGR (% day^{-1})	0.23±0.02	0.18±0.02	0.39±0.01*	0.37±0.01*	0.80±0.04	0.72±0.05
FCE	–	–	1.12±0.00*	1.10±0.00*	–	–
PER	–	–	2.2±0.0*	1.9±0.0*	–	–

The differences in growth and trypsin specific activity in Atlantic salmon possessing different trypsin genotypes were shown to be due to their differences in protein digestion and food utilization under varying environmental conditions. An *in vitro* protein digestibility study by Bassompierre et al. [1998] using pyloric caecal dialyzed crude enzyme extracts from Atlantic salmon with the trypsin genotypes *TRP-2*100/100*, *TRP-2*100/92* and *TRP-2*92/92* indicated distinctive digestion characteristics among them for the same fishmeals (Figure 5). The genotype *TRP-2*100/100* was less able to digest a low quality fishmeal with 86% mink digestibility compared to the *TRP-2*100/92* and *TRP-2*92/92* genotypes, while all trypsin genotypes could well utilize a high quality fishmeal with 94% mink digestibility. The heterozygote *TRP-2*100/92* was the most efficient trypsin genotype in its ability to degrade any type of fishmeals and with a relatively higher liberation potential of free amino acids than the other genotypes (Figure 5). This indicated the advantage of possessing diverse trypsin isozymes, which was confirmed in spiny lobster, *Panulirus argus*, by Perera et al. [2010]. Between the two homozygote genotypes, the *TRP-2*100/100* salmon was more sensitive to feed qualities than the *TRP-2*92/92* genotype (Figure 5). This meant the *TRP-2*92/92* salmon should have better ability for food utilization, and this should result in a higher growth rate than the *TRP-2*100/100* salmon at the rearing condition studied.

A higher feed utilization through digestion ability was also observed in *TRP-2*92* Atlantic salmon (*TRP-2*100/92* and *TRP-2*92/92* genotypes), by studying the facilitation of free amino acids in the plasma and white muscle after a single feeding, compared to those lacking trypsin variant TRP-2*92 (Torrissen et al. 1994). One of the observations was the higher increase in the levels of post-prandial free lysine in the plasma of the *TRP-2*92* salmon, which indicates a higher feed digestion and absorption in these fish, as lysine is one of the amino acids in proteins hydrolyzed by trypsin. Trypsin specific activity in the pyloric caeca decreased during the whole time course and showed lower values in the *TRP-2*92* salmon than the other genotype. These were observed in both 100 g salmon at growing phase and 400 g salmon at steady growth phase. The characteristics of better digestion and absorption

of dietary protein were observed in Atlantic salmon with the trypsin variant TRP-2*92, regardless of whether the fish consumed the feed for growth or for maintenance, compared to the salmon lacking the trypsin variant TRP-2*92.

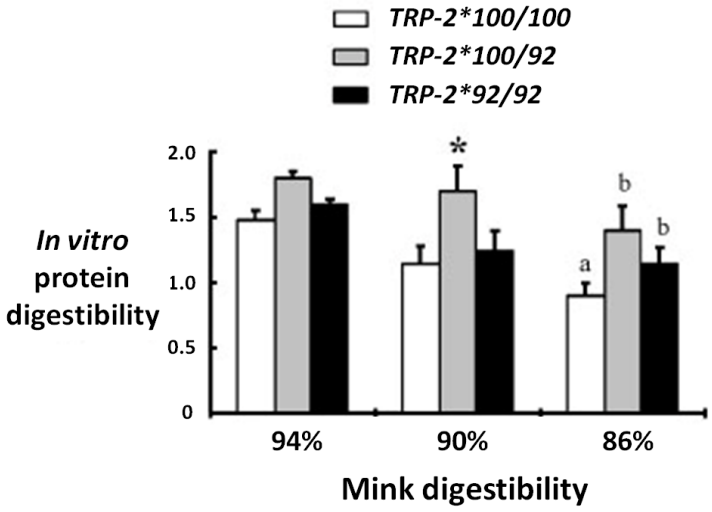

Figure 5. *In vitro* protein digestion potential (expressed as amino group liberation in 10^{-4} mol alanine equivalent) of the pyloric caecal crude enzyme extracts from Atlantic salmon possessing different trypsin genotypes (see Figure 1), using fish meals as substrates with different quality based on mink digestibility. The bars with the asterisk (*) or with different superscripts are significantly different ($P < 0.05$). (Adapted from Rungruangsak Torrissen and Male [2000], with permission from CRC Press LLC, based on Bassompierre et al. [1998]).

The lower trypsin specific activity in the pyloric caecal tissue in the fish with higher feed digestion and absorption by Torrissen et al. [1994] confirmed the earlier finding in the fish with higher growth rate (Torrissen 1987; Figure 2). This could be due to higher secretion of trypsin into the pyloric caecal lumen (Rungruangsak Torrissen and Male 2000). Moreover, trypsin specific activities in the pyloric caecal content were much higher (5–33 times) than those in the pyloric caecal tissue (Torrissen et al. 1994). Therefore, the trypsin specific activity remained lower in the pyloric caecal tissue of the fish with higher growth rate and with higher digestion and absorption of dietary protein.

The comparisons in protein digestion, absorption and transport in salmon with and without the variant TRP-2*92 were further studied (Torrissen et al. 1994). Significantly higher levels of total free amino acids (TFAA) and essential free amino acids (EAA) in plasma and white muscle of the *TRP-2*92* salmon were observed and indicated better protein digestion, absorption and transport in *TRP-2*92* genotype. The transports of TFAA, especially EAA as well as non-essential free amino acids (NEAA), to the white muscle for protein synthesis were faster with higher elevations of almost all FAA in the *TRP-2*92* salmon. The concentrations of free amino acids (FAA) in the plasma and white muscle changed after feeding and they were sustainable to the prefed (PF) values during 48–72 h post-feeding. This indicated an efficiently regulated mechanism for FAA for which the change in concentrations would not be detectable in continuously fed salmon.

Further studies (Torrissen et al. 1995) on the effects of different dietary qualities on protein digestion, and absorption and transport of FAA in salmon with the different trypsin genotypes are shown in Figure 6 and Table 4. Regardless of genotypes, the elevations of FAA in the plasma were detected immediately after feeding (0 h), with the EAA/NEAA ratio values higher for the feed containing partially pre-hydrolyzed protein than the feed containing highly pre-hydrolyzed protein. The qualities of the two experimental feeds are shown in Table 4. The characteristics of the EAA/NEAA ratio in the plasma peaked after 12 h of feeding (Figure 6). The indications of higher feed utilization were seen in salmon fed the partially pre-hydrolyzed than the highly pre-hydrolyzed dietary protein, as higher transports to white muscle of EAA and EAA/NEAA ratio were observed regardless of genotype (Figure 6). The *TRP-2*92* salmon fed the diet containing partially pre-hydrolyzed protein had faster and higher elevations of TFAA, EAA and NEAA (Figure 6) with higher growth rate and with a relatively higher apparent digestibility coefficient (ADC) of protein (Table 4) than the salmon lacking the variant TRP-2*92 fed the same feed. Significant differences in growth rate and absorption of FAA between trypsin genotypes without significant differences in the ADC of protein indicate the ADC method is not sensitive, as earlier finding (Table 3). There were no differences in the elevations of FAA (Figure 6) and in growth rates (Table 4) between the two genotypes fed on highly pre-hydrolyzed dietary protein although the *TRP-2*92* salmon showed a relatively lower ADC of protein (Table 4). Among amino acids, cysteine seemed to have lowest ADC value (Table 4). The productive fat value (PFV) was lower in salmon fed the diet containing partially pre-hydrolyzed than highly pre-hydrolyzed protein (Table 4). This should have led to a higher productive protein value (PPV) in the fish fed the partially pre-hydrolyzed dietary protein, but the PPV seemed to increase with the degree of pre-hydrolysis of dietary protein (Table 4). This controversial PPV may be due to the Kjeldahl method used for which the protein is not directly determined but calculated from nitrogen level.

Moreover, plasma lysine levels were higher in the salmon fed the partially pre-hydrolyzed than highly pre-hydrolyzed protein, and the levels were higher in the *TRP-2*92* salmon than the other genotype without the variant (Torrissen et al. 1995). Since lysine is involved in the peptide bonds hydrolyzed by trypsin, the higher plasma lysine levels could be affected by the quality of the dietary protein and also by the variant TRP-2*92 (Torrissen et al. 1994, 1995). In white muscle, the levels of some amino acids (glutamic acid, glutamine, glycine, alanine, β-alanine, taurine, anserine) involving in protein synthesis (Torrissen et al. 1995) and the levels of free hydroxyproline were significantly higher in the *TRP-2*92* salmon than in the salmon without the variant, especially when feeding on partially pre-hydrolyzed protein (Figure 6). Higher levels of muscle free hydroxyproline in higher growth fish suggests higher metabolism of collagen for remodeling of growing tissues in the muscle, as hydroxyproline is the product of protein breakdown. The lower levels of plasma free hydroxyproline in higher growth fish (Figure 6) suggest lower mobilization of collagen as an energy source. The *TRP-2*92* salmon have higher protein growth efficiency than the salmon without the variant, and the quality of partially pre-hydrolyzed protein is better than highly pre-hydrolyzed protein. The levels of free hydroxyproline in the plasma and white muscle can be reliable indices for growth and nutritional status of the fish (Torrissen et al. 1994, 1995).

Protein synthesis cannot proceed unless all of the constituent amino acids are present and it is limited to the concentration of EAA. The initial increases of FAA in plasma and white muscle are the results of absorption and transport of nutrients from the diets, while the prolonged elevations could also be the results of body protein breakdown. Nutritional status

of Atlantic salmon was better when feed with partially pre-hydrolyzed protein than highly pre-hydrolyzed protein. Differences in protein quality, due to variations in the degree of pre-hydrolysis, affected digestion of protein and utilization of amino acids in Atlantic salmon. Partially pre-hydrolyzed dietary protein with higher contents of peptides having molecular weight more than 66,000 Da (Table 4) promoted higher utilization and growth rates with lower fat deposition, especially in *TRP-2*92* salmon.

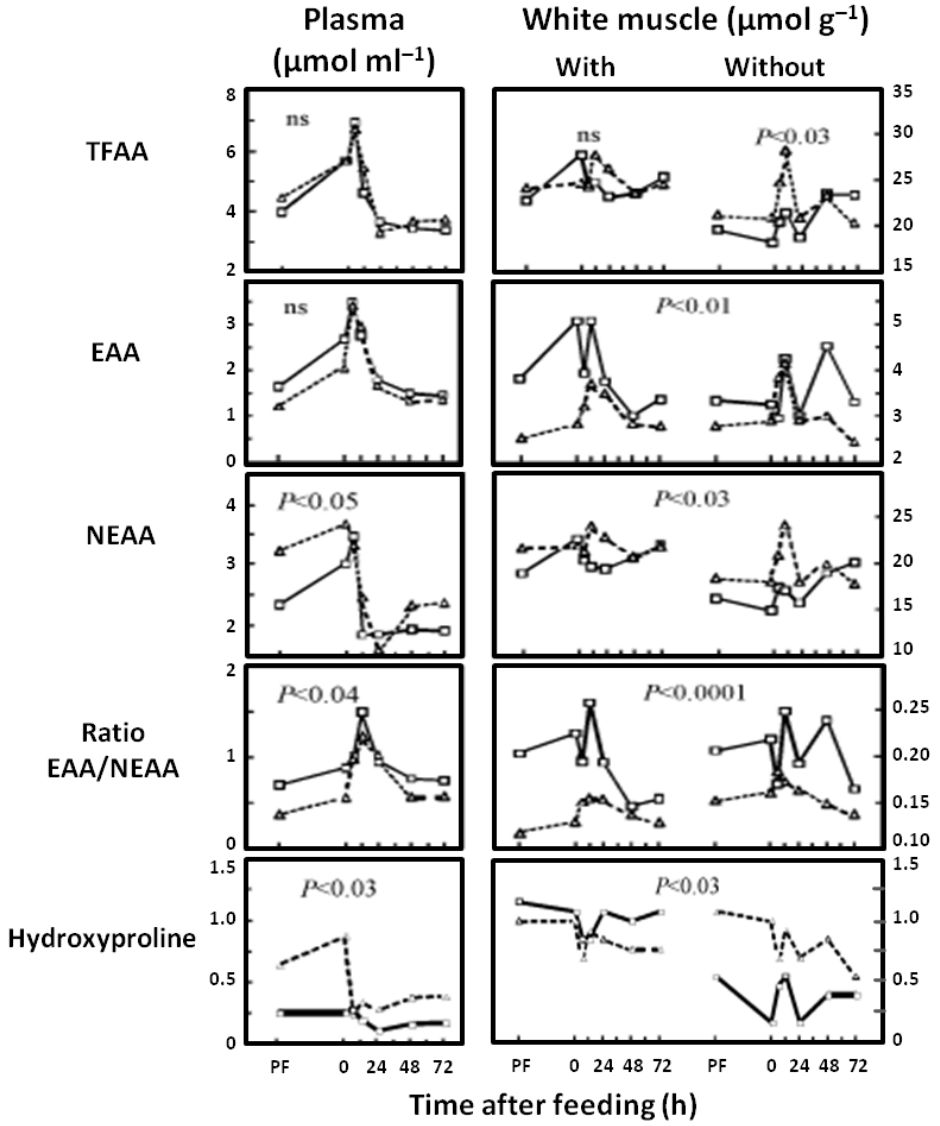

Figure 6. Post-prandial total free amino acids (TFAA), essential (EAA) and non-essential (NEAA) free amino acids, EAA/NEAA ratio, and free hydroxyproline, in the plasma and epaxial white muscle of 100 g Atlantic salmon with and without trypsin variant TRP-2*92 fed on the feeds with partially pre-hydrolyzed protein (—□—) or with highly pre-hydrolyzed protein (---Δ---). Probability values indicate significant differences between the two feed types by paired analysis during the whole time course. PF, prefed values after 2 days starvation; ns, not significant. (Adapted data from Torrissen et al. [1995]).

Table 4. Compositions on dry weight basis, molecular weight (MW) distributions of the dietary protein, total amino acids (TA), peptide amino acids (PA) and free amino acids (FA) of the experimental feeds; and apparent digestibility coefficient (ADC) of amino acids and protein, specific growth rate (SGR), productive protein value (PPV) and productive fat value (PFV) in Atlantic salmon with and without trypsin variant TRP-2*92 fed with two different experimental feeds. The values with asterisk (*) are significantly different between the two genotypes ($P < 0.03$). (Adapted from Torrissen et al. [1995])

Analytical values	Feed containing partially pre-hydrolyzed protein					Feed containing highly pre-hydrolyzed protein				
Dry matter (%)	95.0					94.9				
Protein (%)	50.1					48.9				
Lipid (%)	15.9					15.9				
Ash (%)	6.6					6.3				
MW distribution (% of protein)										
< 2,000	29.8					26.9				
2,000 – 25,000	13.7					35.9				
25,000 – 66,000	0.3					0.1				
> 66,000	56.2					37.1				
Amino acids	Feed quality			Salmon genotypes		Feed quality			Salmon genotypes	
	Concentration (% of protein)			% ADC of individuals		Concentration (% of protein)			% ADC of individuals	
	TA	PA	FA	With	Without	TA	PA	FA	With	Without
Aspartic acid	10.5	3.21	0.02	93.6	91.7	11.0	3.51	0.02	92.1	90.8
Glutamic acid	17.0	5.45	0.09	97.7	96.8	17.5	5.85	0.08	96.4	95.3
Hydroxyproline	1.0	0.63	0.01	96.7	93.2	1.0	0.63	0.01	88.6	90.9
Serine	4.9	1.53	0.02	93.4	92.0	5.1	1.66	0.02	92.1	92.4
Glycine	6.0	2.74	0.04	96.2	95.1	6.2	2.81	0.03	93.2	93.7
Histidine	2.5	1.03	0.00	95.5	94.8	2.6	1.12	0.00	100.0	94.2
Arginine	7.0	2.40	0.01	97.0	95.6	7.3	2.60	0.01	95.3	96.0
Threonine	4.9	1.33	0.02	95.1	92.7	5.1	1.45	0.02	91.5	93.4
Alanine	6.6	2.41	0.04	96.6	94.9	6.8	2.57	0.04	94.0	94.8
Proline	4.7	1.81	0.02	95.5	93.6	4.9	1.91	0.01	89.4	93.2
Tyrosine	3.9	0.78	0.01	95.8	93.5	4.2	1.30	0.02	93.9	93.9
Valine	5.2	1.37	0.01	96.3	94.6	5.4	1.51	0.01	94.0	94.5
Mathionine	3.4	0.91	0.02	97.2	95.6	3.5	1.01	0.01	94.8	95.8
Cysteine	0.1	0.02	0.01	84.6	74.2	0.2	0.01	0.02	46.9	86.9
Isoleucine	5.0	1.24	0.01	96.0	93.9	5.2	1.29	0.01	92.1	93.9
Leucine	9.0	2.33	0.02	96.7	95.1	9.2	2.53	0.03	95.1	94.9
Phenylalanine	4.3	0.98	0.02	96.2	94.4	4.5	1.08	0.02	94.6	94.6
Lysine	10.0	3.89	0.03	98.5	95.7	10.3	4.30	0.03	94.9	96.5
% ADC of protein	–			95.4	93.0	–			89.9	93.2
SGR (% day^{-1})	–			0.72*	0.51*	–			0.52	0.52
% PPV	–			35.75		–			47.13	
% PFV	–			–6.69		–			5.52	

Tryptophan was not analyzed.

A high absorption of FAA in the plasma is not always due to a high quality feed. A low quality feed can also cause a high absorption of plasma FAA, but the transport of FAA to the muscle may not be efficient due to amino acid imbalance as observed in the highly pre-hydrolyzed dietary protein (Figure 6). Therefore the addition of amino acids into a feed may increase plasma amino acids, but they may not be transported efficiently to the white muscle for protein synthesis and growth.

Differences in food digestion, absorption and transport (due to either dietary quality or genetics feature of the fish) that affect the rates and levels of FAA in plasma and white mucle will cause variations in nitrogen metabolism and growth. Higher quality diets (with higher contents of high molecular weight peptides) will cause rapid increases with higher elevations of EAA in plasma and white muscle rather than prolonged elevations, and will promote growth with lower lipid deposition. Salmon possessing the trypsin variant TRP-2*92 will perform better than those lacking this variant at $\leq 8\ ^{o}C$, especially below $6\ ^{o}C$.

Trypsin Genotypes, Free Amino Acid Absorption, Insulin Secretion and Growth

Growth is influenced by genetic variations in trypsin phenotypes through efficiencies in digestion of dietary protein, and absorption and transport of FAA to target tissues, such as white muscle, for synthesis and deposition of protein. Protein metabolism in fish is influenced by anabolic hormone insulin (Inui et al. 1975; Ince and Thorpe 1978; Machado et al. 1988), which stimulates growth in different fish species (Ablett et al. 1981; Sundby et al. 1991).

Rungruangsak-Torrissen and Sundby [2000] observed high sharp peaks of plasma FAA around 8 h post-feeding in *TRP-2*100/92* salmon prior to the high insulin peak. FAA levels are higher in growing tissues than in quiescent tissues (Love 1980), and the incorporation of FAA into body protein during protein synthesis will remove the FAA as fast as they are absorbed (Coulson et al. 1987). Thus, protein synthesis in white muscle should occur around 8 h after feeding in higher growth salmon. This is similar to the observation of a significant increase in muscle protein synthesis rate in Atlantic salmon 9 h after feeding (Fauconneau et al. 1989). Faster responses in plasma insulin levels and the activity ratio of trypsin to chymotrypsin (T/C ratio) in pyloric caeca with food content at ≤ 5 h post-feeding were also observed in the *TRP-2*100/92* salmon. In salmon lacking the trypsin variant TRP-2*92, there were no sharp peaks in plasma FAA profiles during 5–9 h post-feeding, and it took ≥ 6 h for plasma insulin levels to peak after feeding. Small peaks of plasma FAA seemed to be associated with the fluctuation in plasma insulin, and showed that elevations of plasma FAA always occurred prior to the elevations of plasma insulin. Although plasma insulin level was also high around 8 h post-feeding in salmon lacking the variant, amino acid levels were not highly elevated. This suggests a lower rate of protein synthesis in these salmon compared to the *TRP-2*100/92* salmon. The average levels of plasma ratio of essential to non-essential free amino acids (E/N ratio) were similar between the different genotypes (Rungruangsak-Torrissen and Sundby 2000; Rungruangsak-Torrissen 2012).

During routine feeding, increased plasma insulin secretion was associated with increased plasma TFAA levels, especially EAA. During 5–9 h post-feeding, the T/C ratios in the pyloric caeca were negatively correlated with the E/N ratio in the plasma, regardless of genotypes. At the same time, the relationship between the T/C ratios and plasma insulin levels

was only observed in the *TRP-2*100/92* salmon, due to higher protein digestion and FAA absorption to stimulate the secretion of plasma insulin in these fish. No correlation was observed between the E/N ratios and plasma insulin levels in the *TRP-2*100/92* salmon during routine feeding as well as 5–9 h post-feeding (Rungruangsak-Torrissen and Sundby 2000), due to the faster and higher rate of amino acid transport, especially for EAA, to the white muscle for protein synthesis (Torrissen et al. 1994, 1995). There was also no correlation between TFAA and insulin levels in the plasma at 5–9 h post-feeding. All correlations were mainly at 5–9 h post-feeding and seldom during routine feeding (Rungruangsak-Torrissen and Sundby 2000; Torrissen et al. 1994). This indicates a highly physiological control of plasma amino acids pool during regular feeding. Significant relationships between digestion rate of dietary protein, absorption and transport rate of amino acids, and plasma insulin concentration were observed. These relationships were associated with and primarily affected by genetic variations in the expression of different isozymes of trypsin (primary key enzyme for food utilization and growth), and the salmon with the trypsin variant TRP-2*92 performed better than those lacking the variant at the condition studied (Rungruangsak-Torrissen and Sundby 2000; Rungruangsak-Torrissen 2012).

Protein synthesis occurs 8–9 h post-feeding. A high FAA absorption accompany with insulin secretion suggests a higher rate of protein synthesis as observed in the *TRP-2*100/92* genotype with a high growth efficiency, and insulin secretion is stimulated by the elevation of plasma FAA. The results indicate that digestion efficiency of dietary protein (indicated by the pyloric caecal T/C ratio), absorption and transport rate of amino acids (suggested by plasma TFAA and E/N ratio) and plasma insulin level are correlated. These relationships may not be seen if genetic variation in feed utilization is not included in the experimental design.

Trypsin Genotypes, Maintenance Ration, Protein Synthesis Capacity and Insulin Secretion

The associations between dietary protein digestion, absorption and transport of amino acids, and plasma insulin levels, with variations between different trypsin genotypes in Atlantic salmon have been observed (see previous section). This led to studies on how fish growth related to feed consumption rate, protein synthesis capacity in the white muscle, and plasma insulin concentrations, as well as feeding hierarchy, between Atlantic salmon possessing (*TRP-2*100/92* and *TRP-2*92/92*) and lacking (*TRP-2*100/100*) the trypsin variant TRP-2*92 (Rungruangsak-Torrissen et al. 1999a).

During starvation and restricted rations, an advantage on feed utilization was observed in the *TRP-2*92* salmon. The relationships between weight specific consumption rate and specific growth rate (SGR) of individuals were significant in both genotypic groups with similar slopes, whereas the significant differences in the elevation of the two regressions resulted in a lower maintenance ration (at SGR = 0) of the salmon with *TRP-2*92* genotype (0.11% body weight day^{-1}) than the other group lacking the variant (0.13% body weight day^{-1}). There was also a correlation between weight specific consumption rates and plasma insulin levels, regardless of trypsin genotypes (Rungruangsak-Torrissen et al. 1999a).

Table 5. Analytical results (mean±sem) during starvation and growing periods of Atlantic salmon smolts (80 g) with and without trypsin variant TRP-2*92 at 9.4±0.1 °C with salinity 17.2±0.3. Within the same row, the values with different superscripts are significantly different ($P < 0.05$). (Adapted from Rungruangsak-Torrissen et al. [1999a], with permission from Springer Corp.)

Analytical values	With TRP-2*92		Without TRP-2*92	
After 2 weeks starvation				
Specific growth rate (% day^{-1})	–0.28 ± 0.08		–0.25 ± 0.03	
Muscle RNA (µg mg $muscle^{-1}$)	0.19 ± 0.02[a]		0.14 ± 0.01[b]	
Muscle Protein (mg mg $muscle^{-1}$)	0.12 ± 0.01		0.13 ± 0.01	
Muscle RNA/Protein (µg mg^{-1})	1.64 ± 0.14[a]		1.12 ± 0.12[b]	
Protein synthesis rate (% day^{-1})	0.15 ± 0.02		0.12 ± 0.01	
RNA activity (g protein synthesized g RNA^{-1} day^{-1})	0.98 ± 0.15		1.15 ± 0.12	
Feeding rate (% body weight day^{-1})	0.5	1	0.5	1
Day 58				
Consumption rate (% body weight day^{-1})	0.20±0.02	0.19±0.02	0.21±0.02	0.20±0.03
Specific growth rate (% day^{-1})	0.18±0.05	0.14±0.03	0.15±0.05	0.12±0.07
Day 164				
Specific growth rate (% day^{-1})	0.40±0.02[a]	0.53±0.02[b]	0.38±0.03[a]	0.54±0.04[b]
Plasma insulin (ng ml^{-1})	15.57±1.67	16.20±1.48	14.13±1.07[a]	17.67±1.39[b]
Day 190				
Specific growth rate (% day^{-1})	0.66±0.07[a]	0.77±0.09[a]	0.47±0.09[b]	0.79±0.09[a]

During 58 days of feedings, neither weight specific consumption rates nor specific growth rates were different between the two genotypic groups (Table 5). When the SGRs were significantly different between feeding rates on day 164, regardless of trypsin genotypes, only the salmon lacking the variant showed differences in plasma insulin levels and the fish fed at 1% of body weight day^{-1} had higher levels than those fed at 0.5% of body weight day^{-1}, and these fish showed differences in SGR between different feeding rates until the end of the experiment on day 190. Contrastingly, in the *TRP-2*92* genotypic group, the SGRs that were different between feeding rates on day 164 became similar one month later on day 190. The similar plasma insulin levels between feeding rates on day 164 in the *TRP-2*92* genotype may reflect the similar growth rates one month later. This suggests an advantage of lower maintenance ration of the salmon with *TRP-2*92* genotype on feed utilization at restricted ration through increasing plasma insulin at 0.5% feeding rate to a similar level as 1% feeding rate before similar growth rates were observed (Table 5).

Increased trypsin specific activity accompanied with increased plasma insulin levels occurred at least one month before enhanced growth rates were observed (Rungruangsak-Torrissen et al. 1999a; Rungruangsak-Torrissen 2012). This confirms the reason why the plasma insulin levels in the *TRP-2*92* salmon with different feeding rates were similar on day 164, one month before their different growth rates became similar on day 190 (Table 5). These results also confirm the digestion of dietary protein by trypsin as the primary mechanism, followed by plasma insulin secretion, for protein synthesis and growth in Atlantic salmon. During starvation, the levels of RNA in the white muscle were significantly

higher in the *TRP-2*92* salmon, resulting in higher muscle ratio of RNA/protein (Table 5). This indicates a higher capacity for protein synthesis in *TRP-2*92* salmon, compared to those lacking the variant.

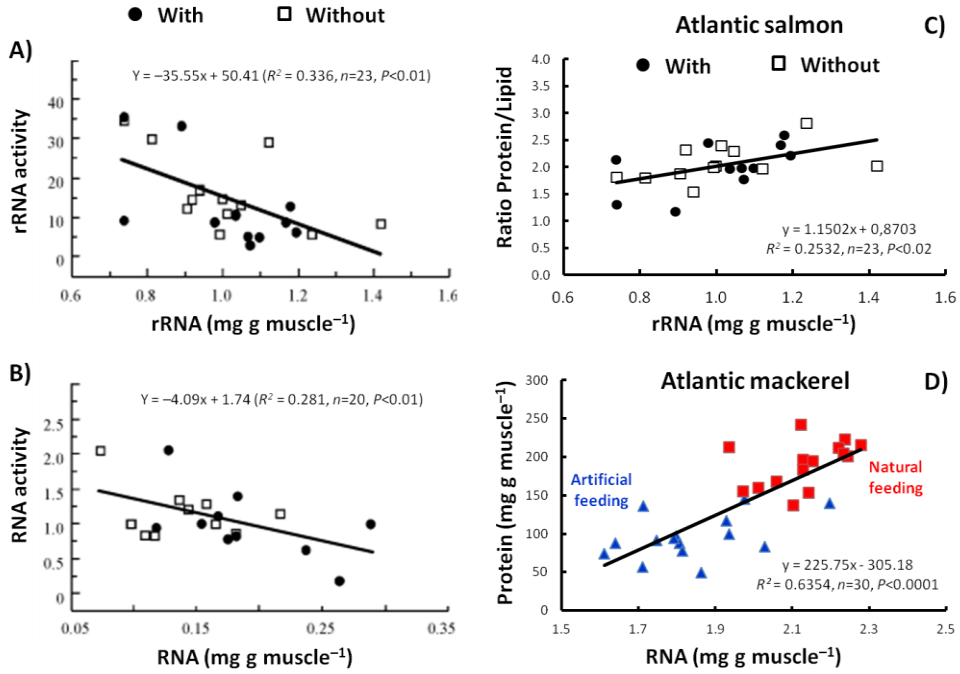

Figure 7. The relationships in Atlantic salmon with and without the trypsin variant TRP-2*92, (A) between rRNA activity (pmol ^{14}C-phenylalanine mg rRNA^{-1}) in the *in vivo* protein synthesis and rRNA concentrations in the white muscle, (B) between RNA activity (g protein synthesized g RNA^{-1} day^{-1}) in the *in vivo* protein synthesis and RNA concentrations in the white muscle, and (C) between rRNA concentrations and the composition ratio of protein to lipid in the white muscle. (D) The relationship in Atlantic mackerel with different feedings, between RNA concentrations and protein levels in the white muscle. (Adapted data (A and B) from Rungruangsak Torrissen and Male [2000], with permission from CRC Press LLC, and (C and D) from Rungruangsak-Torrissen and Fosseidengen [2007], with permission from John Wiley and Sons, Inc.).

Protein synthesis rates were similar between the two genotypic groups, and a trend of lower RNA activity was observed in the *TRP-2*92* salmon (Table 5). The activity levels of both ribosomal RNA (rRNA) (Figure 7A) and RNA (Figure 7B) in the white muscle were negatively correlated with their concentrations, regardless of trypsin genotypes. However, increased concentrations of rRNA and RNA were correlated with the increased protein levels, as observed between the concentrations of rRNA and the ratio of protein/lipid in the white muscle of Atlantic salmon (Figure 7C), and between the concentrations of RNA and protein in the white muscle of Atlantic mackerel, *Scomber scombrus* L. (Figure 7D).

These results indicate the possibility that the *TRP-2*92* salmon, which had low maintenance ration, had higher capacity for protein synthesis and maintained higher RNA concentrations in the white muscle during starvation. They were more sensitive to changes in feed intake, for which feed utilization would favour protein synthesis and turnover where higher deposition of protein would be observed in these salmon, compared to those lacking

the trypsin variant TRP-2*92 feeding on the same diet. The *TRP-2*92* salmon could be defined as a high protein growth efficiency fish with low protein turnover rate. No feeding hierarchy was observed between the different trypsin genotypes at restriced ration (Rungruangsak-Torrissen et al. 1999a).

Trypsin Genotypes and Immune Responses

Genetic variations in trypsin genotypes due to variations in expression of different trypsin isozymes in the pyloric caeca were illustrated in the previous sections. These affect on growth through digestion of dietary protein, absorption and transport of amino acids (especially essential amino acids), plasma insulin secretion, and capacity for protein synthesis in white muscle. Atlantic salmon with *TRP-2*92* genotype (*TRP-2*100/92* and *TRP-2*92/92*) show better growth performance than the salmon lacking the variant at $\leq 8\ ^{o}C$, especially below $6\ ^{o}C$. In fish with higher growth rate, health is always in question if they will be selected for aquaculture production. This led to investigations on differences in immune parameters and in disease resistance between groups possessing and lacking the trypsin variant TRP-2*92 (Rungruangsak-Torrissen et al. 1999b).

Unvaccinated Atlantic salmon were infected with furunculosis caused by *Aeromonas salmonicida* ssp. *salmonicida*. After 48 days with two outbreaks and two medications, there were no statistical differences in resistance to furunculosis or in response to medication between the two fish groups possessing and lacking the trypsin variant TRP-2*92. The total cumulative mortalities were 85% and 89%, respectively. In addition, a cohabitant challenge test was performed in smolts intraperitonially injected with *A. salmonicida*. After 23 days of challenge, there were no statistical differences in mortality between the unvaccinated smolts with (84%) and without (76%) the trypsin variant TRP-2*92. The 8% difference in cumulative mortality was the same as the difference between the infected cohabitants of the two fish groups. Also, unvaccinated Atlanic salmon with different trypsin genotypes had similar resistance to furunculosis and in response to medication (Rungruangsak-Torrissen et al. 1999b).

Further studies were conducted to investigate differences in specific and non-specific immune parameters in Atlantic salmon with different trypsin genotypes after vaccination (Rungruangsak-Torrissen et al. 1999b). The fish were cultured together and vaccinated with a non-adjuvanted vaccine against furunculosis. Four weeks after vaccination, there was a significant negative correlation between total serum IgM and fish weight, regardless of trypsin genotypes. Within the same weight range of 80–170 g, the *TRP-2*92* Atlantic salmon showed significantly higher total serum IgM ($345\pm30\ \mu g\ ml^{-1}$) than the salmon without the variant ($236\pm18\ \mu g\ ml^{-1}$) four weeks after vaccination. The result also indicates that vaccination should be performed in salmon of about 100 g in order to get a high IgM response.

Rungruangsak-Torrissen et al. [1999b] also performed another vaccination experiment with a commercial multiple vaccine, a glucan and oil adjuvanted multiple vaccine against furunculosis, vibriosis, cold water vibriosis, and IPN (Intervet Norbio A/S, Norway). Post-smolts of each genotype were cultured separately and studied the responses of different immune parameters five months after vaccination with the multiple vaccine. The responses of the specific antibodies against *A. salmonicida* to the multiple vaccine were slightly different

between salmon with and without the trypsin variant TRP-2*92 but the values were not statistically different. For non-specific immune responses, there were some differences. Except for total serum complement haemolytic activity of CH50 which was similar, the specific levels (per mg serum protein) of total serum IgM and lysozyme were significantly lower in the salmon with homozygote *TRP-2*92/92* genotype, while the total serum complement haemolytic activity of SH50 was higher, compared to the *TRP-2*100/100* genotype without the variant. The heterozygote *TRP-2*100/92* genotype showed alternative responses, either similar to *TRP-2*92/92* genotype in specific level of total serum IgM or to *TRP-2*100/100* genotype in total specific activities of SH50 and lysozyme.

Total serum protein concentration, total specific activity of CH50, and total serum IgM were not to be affected by vaccination (Melingen et al. 1995). Therefore, the differences in total serum IgM between trypsin genotypes indicate the effects of genetic variation in expression of different trypsin isozymes on immune responses after vaccination. There was a negative genetic correlation between specific activities of lysozyme and SH50 (Røed et al. 1993; Rungruangsak-Torrissen et al. 1999b). The levels of total serum IgM in *TRP-2*92* salmon were higher 4 weeks after vaccination and became lower 5 months after vaccination, compared to the salmon without the variant. This was probably due to a higher capacity for protein synthesis (Rungruangsak-Torrissen et al. 1999a) with a more rapid immune response in *TRP-2*92* salmon after vaccination. Moreover, the kinetics of antibody production might be different among the trypsin genotypes or the type of vaccine.

Dietary nutrients are able to affect immune system (Waagbø 1994), and optimal dietary protein concentration (Kiron et al. 1993) as well as the amino acid profile in the diet (Neji et al. 1993) is important for disease resistance. In addition, trypsinogen genes have been found in the DNA of the human β T cell receptor locus which has a vital role in immunity (Rowen et al. 1996). Trypsin genotypes affect growth (Figures 2–3 and Tables 4–5) through food utilization, and result in physiological changes of amino acid profiles and nutritional status of the fish (Figures 5–6 and Tables 3–5) as well as changes of immune responses after vaccination. Thus trypsin genotypes can indirectly affect the immune system for survival and growth, which are controlled through food utilization, especially through the dietary protein consumed.

Molecular Characterization of Anionic and Cationic Variants of Trypsin

Trypsin is a major digestive enzyme in the large family of serine proteases, and its well known structural information in mammals has made it an excellent protein model for studying the relationship between sequence, structure, and function of different isoforms of trypsin in Atlantic salmon (Male et al. 1995). Trypsin genes in mammals (Craik et al. 1984; Fletcher et al. 1987) have similar structure to chymotrypsin whereas their differences in substrate specificities are due to differences in the substrate-binding pockets, two supporting loops (Hedstrom et al. 1992) and certain contributing amino acid residues (Hedstrom et al. 1994a, 1994b). Different trypsin isoforms with major differences in the distribution of charged amino acids may have different substrate-binding preferences (Craik et al. 1984). Several isoforms of trypsin have also been described in fish (see Rungruangsak Torrissen and Male 2000), which may possess different kinetic properties (Asgeirsson et al. 1989), and cold-adapted fish species display substantially higher catalytic efficiencies than their mammalian counterparts

(Hjelmeland and Raa 1982; Simpson and Haard 1984; Martinez et al. 1988; Asgeirsson et al. 1989; Taran and Smovdyr 1992). Cloning and sequencing of cDNA libraries from pancreatic tissue of Atlantic salmon were performed, with over 100 primary clones isolated and five clones containing near full-length transcripts were characterized (Table 6). Two clones (pSTRP10 and pSTRP1A) appear to contain the entire coding region. Translation *in vitro* of one of the trypsin clones produced a protein with the expected trypsin molecular mass of 24.5 kDa. Three of the Atlantic salmon trypsins (SalTRP-I, SalTRP-IA, and SalTRP-IB) have very similar sequences (although displaying significant differences) and may represent allelic variants encoded by the same gene locus, while two other trypsins (SalTRP-II and SalTRP-III) are more divergent in sequence and probably encoded by separate gene loci. The charged amino acid distributions show four of the trypsin clones encode anionic forms and the fifth clone represents a cationic variant. All residues differing in charge between anionic and cationic forms are located at exposed regions of the proteins (Male et al. 19950.

Table 6. Comparisons of nucleotide and amino acid sequence identities of trypsin variants from Atlantic salmon. (From Male et al. [1995], with permission from John Wiley and Sons, Inc.)

Name of clone	Length (base pairs)	Similarity (%)	Name of protein	Type	Length (amino acids)	Similarity (%)
pSTRP10	862	100	SalTRP-I	Anionic	222	100
pSTRP1A	868	99	SalTRP-IA	Anionic	215	100
pSTRP2	777	87	SalTRP-IB	Anionic	222	96
pSTRP6	826	90	SalTRP-II	Anionic	222	98
pSTRP41	810	68	SalTRP-III	Cationic	223	69

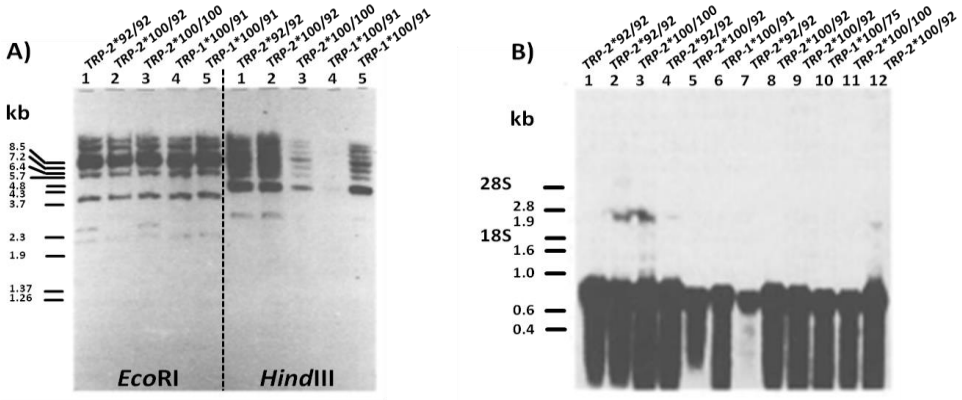

Figure 8. (A) Southern blot of DNA from five individual Atlantic salmon, previously classified to their trypsin isozyme patterns. Each sample contained 10 µg DNA and was digested overnight with 100 U of different restriction enzymes, *Eco*RI and *Hind*III are shown. The migration of a *Bst*EII-digested λ-phage DNA marker is indicated on the left. (B) Northern blot of RNA extracts from pancreatic tissue of individual Atlantic salmon previously assorted according to trypsin isozyme patterns. The Northern blot was hybridized to a STRP41 cDNA probe and exposed to XAR-5 film (Kodac) overnight. The migration of a RNA marker is indicated on the left. Different trypsin genotypes and isozyme patterns are illustrated in Figure 1. (Adapted from Male et al. [1995], with permission from John Wiley and Sons, Inc.).

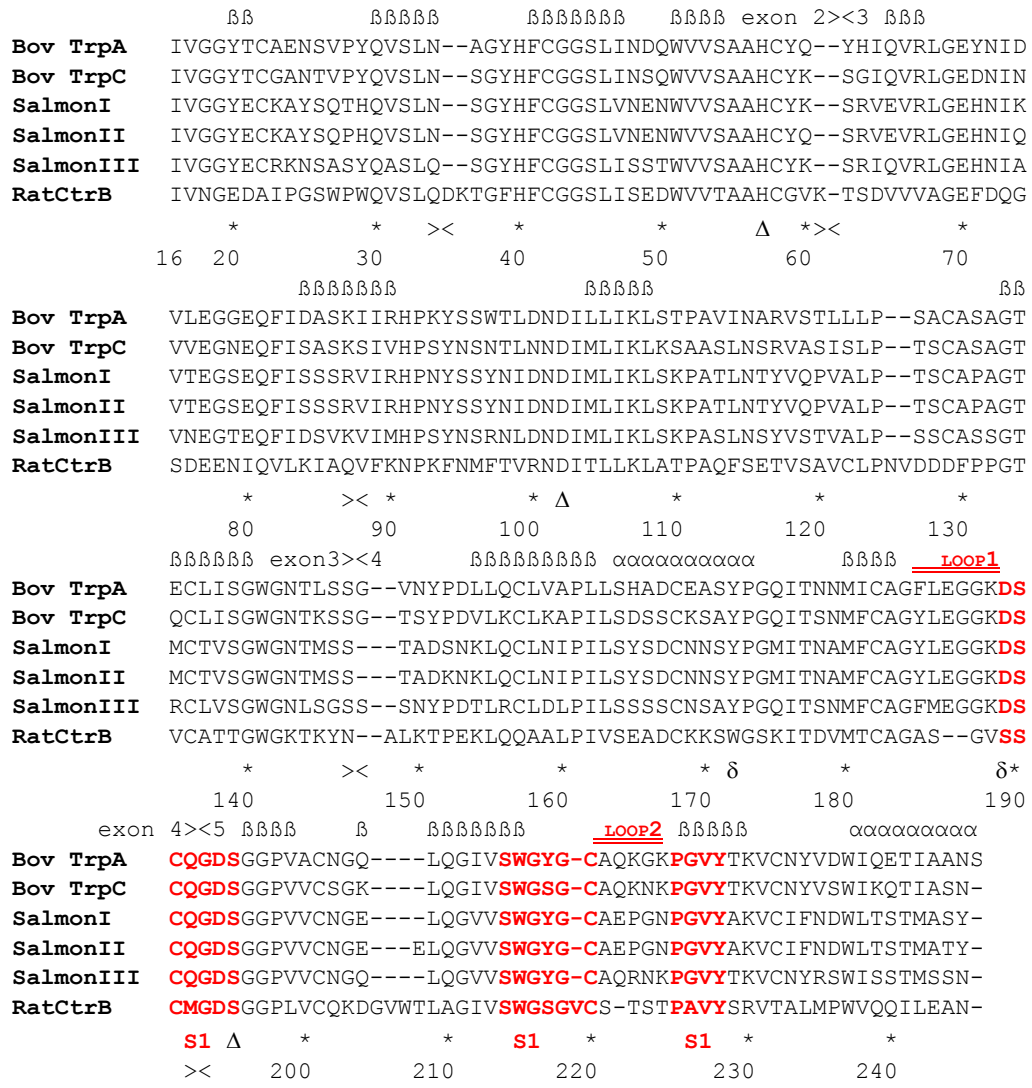

Figure 9. The alignment of amino acid sequences of trypsin variants in Atlantic salmon [SalmonI (SalTRP-I) anionic trypsin I, SalmonII (SalTRP-II) anionic trypsin II, SalmonIII (SalTRP-III) cationic trypsin III], in comparison with Bovine trypsins [*Bos taurus*, anionic (Bov TrpA) and cationic (Bov TrpC) trypsins] and Rat chymotrypsin [*Rattus norvegicus* chymotrypsin B]. The numbers (underneath) refer to the classic system for chymotrypsinogen (Hedstrom et al. 1994b0. The residues in the catalytic triad are indicated by Δ. In accordance with Hedstrom et al. [1992, 1994a, 1994b] the trypsin determinant residues 172 and 189 are marked with δ, the two surface loops (loop 1: residues 184a–188a; and loop 2: residues 221–225) are noted together with residues in the S1 binding pocket. Secondary structures in the salmon trypsin is indicated as α (α-helix) and β (β-sheet) structure according to Smalås et al. [1994]. Exon/intron borders are indicated as > <. (Adapted from Rungruangsak Torrissen and Male [2000], with permission from CRC Press LLC).

Analysis using Southern blotting of genomic DNA from individual Atlantic salmon with different trypsin genotypes indicates a complex pattern of bands with a large number of gene loci for Atlantic salmon trypsin (Figure 8A). Using the restriction enzyme *Eco*RI also revealed a polymorphic DNA band (Figure 8A). Since the *Eco*RI does not cut any of the

salmon trypsin sequences, the polymorphic site is probably situated in intron and/or flanking sequences. Consistent with the length of cDNA clones, the trypsin transcript length analyzed using Northern blotting showed approximately 950b with variations in hybridization signal among individual RNA samples (Figure 8B), which was probably caused by variations in the amount of RNA loaded onto the gel. Northern hybridizations using RNA from individual salmon showed a relatively stable total level of trypsin mRNA. Comparisons of the deduced amino acid sequences of the mature trypsins between mammals and salmon are illustrated in Figure 9. The amino acids generating the substrate-binding pocket are of typical trypsin nature in all salmon sequences, with Asp189 at the bottom, and Gly216 and Gly226 lining the sides of the pocket. Two loops supporting the substrate-binding pocket (Hedstrom et al. 1992) are indicated along with residue 172, a key residue in substrate specificity (Hedstrom et al. 1994a0, whereas loop 1 and residue 172 are conserved in the salmon sequences (Figure 9). All 12 cysteine residues, generating six disulphide bonds, are conserved. The structure of salmon trypsin SalTRP-I (SalmonI) is illustrated in Figure 10. A stereo view of salmon and bovine trypsins with the loop structures connecting to the β-sheets is illustrated in Figure 10A, whereas the main differences between the two species are located in loop region in the crystal structures (Figure 9). The spacefill models of trypsin structure showing charged amino acids (Figure 10B) and hydrophobicity (Figure 10C) are illustrated. The three residues in the catalytic triad (His57, Asp102, and Ser195) are positioned in the junction between the two β-barrels (see Figure 9).

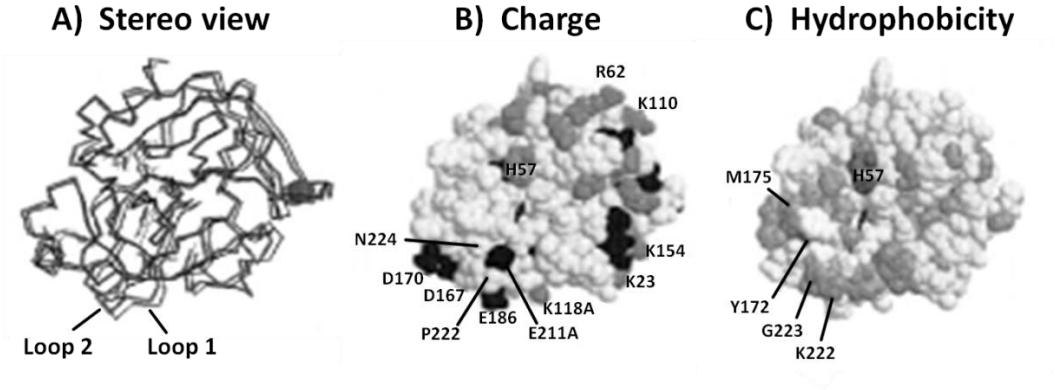

Figure 10. The structure of Atlantic salmon trypsin identical to the SalmonI in Figure 9. (A) Stereo view of salmon (thick lines) and bovine (thin lines) trypsins, with the enzyme associated Ca ion shown as a sphere. Loop 1 and Loop 2 refer to the structures marked in Figure 9. (B) Spacefill model of SalmonI where charged amino acids are marked. Basic residues are light grey and acidic residues are dark grey. (C) Spacefill model of SalmonI where hydrophobic residues are shown in grey. (Adapted from Rungruangsak Torrissen and Male [2000], with permission from CRC Press LLC).

It was difficult to match the trypsin variants observed (Figure 1) with the studies of DNA (Figure 8A) and RNA (Figure 8B) and the trypsin sequences (Figure 9), except for the confirmation of the conservation of trypsin structure which is very similar between salmon and mammalian trypsins. Apparently, there is very little genetic variation in trypsin genes in Atlantic salmon. A fraction of the functional genes seems to be expressed in an individual at a given time and that the pattern of genes that are expressed varies. However, little is known regarding control and which specific genes are expressed.

Phylogenetic analysis of serine proteases has shown that the anionic and cationic trypsins from salmon are equally distant compared to salmon and mammals. This indicates an early separation of the cationic and anionic trypsins during evolution, probably before the fish ramification (Male et al. 1995; Rungruangsak Torrissen and Male 2000).

Effects of Ploidy on Protein Utilization and Growth

The knowledge on the effects of trypsin genotypes on feed utilization and growth was implemented to investigate the effects of ploidy and light regimes on feed utilization and growth in Atlantic salmon (Sunde et al. 2001).

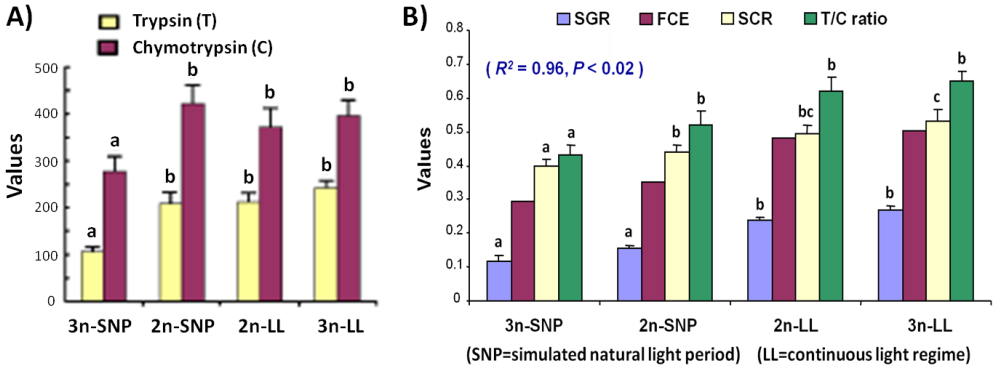

Figure 11. (A) The protease specific activities of trypsin and chymotrypsin (μmol *p*-nitroaniline h^{-1} mg $protein^{-1}$ in the pyloric caeca with food content). (B) The relationships between specific growth rate (SGR: % day^{-1}) with, protease specific activities of trypsin and chymotrypsin (in A), feed conversion efficiency (FCE) on tank basis (statistical analysis could not be performed), specific consumption rate (SCR), and with the protease activity ratio of trypsin to chymotrypsin (T/C ratio). The experiment was performed in diploid (2n) and triploid (3n) Atlantic salmon (about 1 kg), reared for 2.5 months at 9 ^{o}C in seawater tanks under simulated natural light period (SNP) and 24 h light regime (LL). On group basis, the correlation coefficient (R^2) between the SGR, FCE, SCR, and the T/C ratio are significant (indicated in the bracket), regardless of ploidy and light regime. The same parameters with different superscripts are significantly different ($P < 0.05$). (Adapted from Rungruangsak-Torrissen [2002], based on Sunde et al. [2001]).

Variations in protease specific activities of trypsin and chymotrypsin were observed between diploid (2n) and triploid (3n) Atlantic salmon reared under simulated natural light period (SNP) and 24 h light regime (LL) (Figure 11A). Studies from individual salmon showed highly significant relationships among specific growth rate (SGR), feed conversion efficiency (FCE), specific consumption rate (SCR), and the protease activities ratio of trypsin to chymotrypsin (T/C ratio) (Figure 11B), independent of the specific activity levels of trypsin and chymotrypsin (Figure 11A). The 3n-SNP salmon with lowest SGR showed lowest SCR (Figure 11B), with lowest digestive efficiency of protein (T/C ratio) and absorption of amino acids (plasma EAA and TFAA). In spite of similar plasma EAA/NEAA ratio with highest muscle RNA levels and capacity for protein synthesis (RNA/protein ratio) compared to the other fish groups, the 3n-SNP salmon showed lower incorporation of amino acids in the white muscle for protein synthesis indicated by remaining of high level of muscle

EAA/NEAA ratio and the lowest level of muscle protein concentration. The levels of muscle hydroxyproline and SGR were highly correlated, and they were inversely correlated with muscle EAA/NEAA ratio. The appearance of hydroxyproline levels is from the breakdown of collagen and could be an indicator of the catalytic activity in muscle tissues (Torrissen et al. 1994, 1995; Rungruangsak Torrissen and Male 2000). The results confirm the association of muscle hydroxyproline levels with protein growth efficiency in Atlantic salmon.

Increases in SGR had negative relationships with the muscle concentrations of RNA and RNA/protein ratio (Sunde et al. 2001), and increases in RNA levels had negative correlation with its activity which indicates decreases in protein turnover rate (Figure 7B). In addition, high protein growth efficiency is associated with low protein turnover rate (Rungruangsak-Torrissen et al. 1999a). Since growth is affected by both protein and lipid depositions, a tendency of lower protein levels in salmon reared under the continuous light regime (LL) with low protein turnover rate should indicate the effect of continuous light regime on increased growth through increased lipid deposition, compared to the SNP fish groups, regardless of ploidy (Sunde et al. 2001). Moreover, continuous light regime affected higher SGR through significantly higher SCR in triploid (3n) salmon but not in diploid (2n) salmon (Figure 11B). This indicates the diploid salmon have better feed utilization than the triploid fish under the same living condition and with similar feeding rates.

The T/C ratio level in the pyloric caeca is associated with growth and feed efficiency, regardless of protein or lipid growth (Figure 11B). The four fish groups could be ranked similarly as 3n-LL > 2n-LL > 2n-SNP > 3n-SNP with regards to T/C ratio as well as SGR, FCE, SCR, trypsin specific activity, and muscle hydroxyproline level. The relationship between trypsin and chymotrypsin specific activities were further studied in individual salmon to determine the "slope T/C ratio" (slope of regression line) in comparison with the directly calculated "T/C ratio" (Figure 12). The ranking among the different fish groups by the slope T/C ratio was 2n-SNP > 2n-LL > 3n-LL > 3n-SNP, whereas the 3n-SNP fish group had the lowest values for both T/C ratio and slope T/C ratio (Figure 12) as well as SGR (Figure 11B). The slope T/C ratio indicates fish growth rate at sampling, while the T/C ratio indicates fish growth rate over a period of 1–2 months (Rungruangsak-Torrissen et al. 2006, 2009a).

The continuous light regime will stimulate fish growth during winter to spring when the natural day length is short, but it will reduce fish growth later in summer when the natural day length is long by precedently decreasing the T/C ratio in late spring (Rungruangsak-Torrissen et al. 2009a). The highest slope T/C ratio in late spring of the 2n-SNP salmon (Figure 12) could be due to the salmon preparing for a fast growth, while a negative effect on slower growth had been started in 3n-LL salmon as shown by a lower slope T/C ratio (Figure 12). The 2n-LL salmon had a good ranking for both T/C ratio and slope T/C ratio (Figure 12). The fluctuations in the levels were positive in diploid salmon, but negative in triploid fish. This indicates the advantage of normal diploid over triploid salmon in feed utilization and growth.
The relationships between specific growth rates with different biochemical parameters were studied (Figure 13). The factors that indicate growth rate are trypsin specific activity (Figure 13A) and the T/C ratio (Figure 13B). Protein growth efficiency is indicated by increased free hydroxyproline (Figure 13C) and decreased ratio of EAA/NEAA (Figure 13D) in the white muscle. This is similar to the earlier observations by Torrissen et al. [1994, 1995] (Figure 6 and Table 4). The levels of muscle RNA (Figure 7D) as well as rRNA (Figure 7C) are related with protein deposition levels in the white muscle (Rungruangsak-Torrissen and Fosseidengen 2007). The negative correlations between specific growth rate (SGR) with the

white muscle concentrations of RNA (Figure 14A) and the ratio of RNA/protein (Figure 14B) indicate that the continuous light regime does not stimulate fish growth through increased protein deposition, but instead through increased lipid deposition.

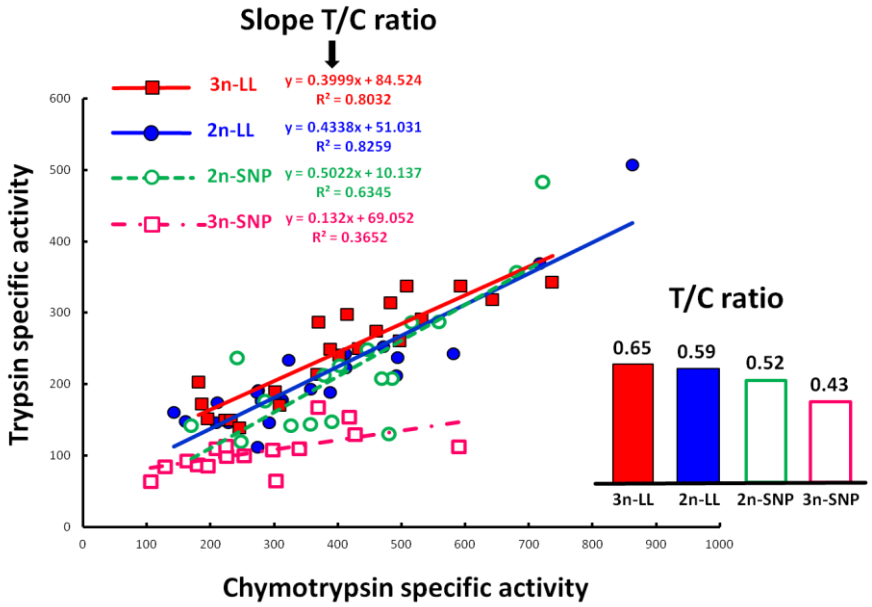

Figure 12. The relationships between specific activities of trypsin and chymotrypsin (expressed as μmol *p*-nitroaniline h^{-1} mg $protein^{-1}$ in the pyloric caeca with food content), and the values of protease activity ratio of trypsin to chymotrypsin (T/C ratio) with significant different value indicated by asterisk (*) on the bar ($P < 0.05$) in comparisons with the slope T/C ratios indicated by the slopes of the regressions. The experiment was performed in diploid (2n) and triploid (3n) Atlantic salmon (about 1 kg), reared for 2.5 months (February–April) at 9 ^{o}C in seawater tanks under simulated natural light period (SNP) and 24 h light regime (LL). (Data were adapted from Sunde et al. [2001]).

A principal component analysis (PCA) of the measured variables explained 80.6% of the variance in the data, regardless of ploidy and light regime (Sunde et al. 2001). Muscle free hydroxyproline showed the highest correlation which explained 55% of SGR variability, while trypsin specific activity and T/C ratio explained 11.5% and 15.2%, respectively (see also from R^2 values in Figure 13). According to the principal component levels, the most direct effects on SGR are the levels of free hydroxyproline and RNA in the white muscle, followed by the levels of EAA and TFAA in the white muscle, then the protease specific activities of trypsin and chymotrypsin, and the levels of EAA and TFAA in the plasma having the least effects on SGR. The levels of protease specific activities of trypsin and chymotrypsin are more practical for growth study, although with low but significant correlation with the SGR (Sunde et al. 2001, 2004; Rungruangsak-Torrissen et al. 2002, 2006, 2009a, 2009b). These proteases do not directly affect growth rate, but they are the key biological factors influencing other biochemical parameters in the growth process (Rungruangsak Torrissen and Male 2000). Studies of different biochemical parameters simultaneously have made it possible to explain a process of growth under different growth statuses, from reduced growth to steady growth and to high growth rates. The direction of

changes in biochemical parameters is dependent on the growth status of the animal which is very important for interpreting the results. A higher growth rate is associated with a higher T/C ratio, and it can be associated with either a higher consumption rate and/or a higher feed utilization. Higher protein growth efficiency is associated with higher levels of RNA and free hydroxyproline in the white muscle.

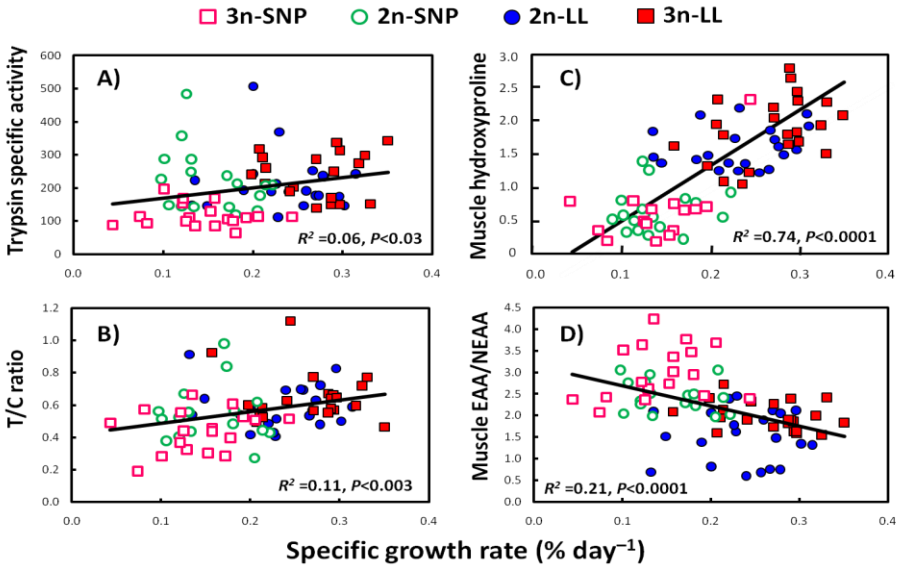

Figure 13. The relationships between specific growth rate (A) with trypsin specific activity expressed as µmol *p*-nitroaniline h^{-1} mg $protein^{-1}$ in the pyloric caeca with food content, (B) with the protease activity ratio of trypsin to chymotrypsin (T/C ratio), (C) with free hydroxyproline concentration (nmol mg^{-1}) in the white muscle, and (D) with the ratio of essential to non-essential free amino acids (EAA/NEAA) in the white muscle. The experiment was performed in diploid (2n) and triploid (3n) Atlantic salmon (about 1 kg), reared for 2.5 months at 9 ^{o}C in seawater tanks under simulated natural light period (SNP) and 24 h light regime (LL). (Data were adapted from Sunde et al. [2001]).

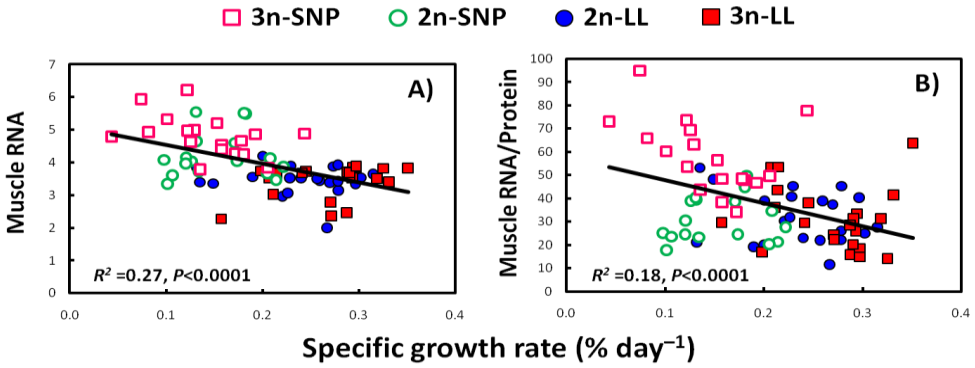

Figure 14. The negative relationships, showing lipid growth, between specific growth rate (A) with RNA concentration (µg mg^{-1}) in the white muscle, and (B) with the ratio of RNA/Protein (µg mg^{-1}) in the white muscle. The experiment was performed in diploid (2n) and triploid (3n) Atlantic salmon (about 1 kg), reared for 2.5 months at 9 ^{o}C in seawater tanks under simulated natural light period (SNP) and 24 h light regime (LL). (Data were adapted from Sunde et al. [2001]).

Should Wild Salmon Populations be Threatened by Escapees?

It is reasonable to conclude that cultured salmon, if they escape, do not threaten wild salmon populations as long as they are healthy and are not genetically manipulated (Rungruangsak-Torrissen 1999, 2002).

Based on studies on genetic variations in trypsin isozyme expression (important key factor for food utilization efficiency and growth) that is affected by environmental changes (such as water temperature and food availability), it is a unique and good example to show that fish can adapt to their environments. Adaptations can be by changing their gene expressions at the molecular and protein levels. Changes at molecular level are by changing gene expression of offspring (Torrissen et al. 1993) and in frequency distribution of trypsin genotypes at different temperatures under egg incubation and start-feeding periods (Rungruangsak-Torrissen et al. 1998; Rungruangsak Torrissen and Male 2000) (Table 2) regardless of trypsin genotypes of parents. Changes at protein level are by changes in specific activities levels of trypsin and chymotrypsin as well as T/C ratio (Tables 1 and 2). This means that gene expression of offspring of escapees and wild salmon can be adapted to their environment. The expression of genes can be switched off and on depending on the environmental conditions, especially at the early life stage. The other adaptation in nature is by migrating to a zone with suitable environmental condition for their genetic feature, especially at an ambient temperature suitable for optimizing efficiency of food utilization and growth, as seen in different temperature distributions in trypsin genotypes of Atlantic salmon in the Norwegian Sea (Rungruangsak-Torrissen and Stensholt 2001; Rungruangsak-Torrissen 2012).

Gene expression is dynamic and genetic structure of any salmon population is also dynamic, and it can occur under both aquaculture and natural ecosystems. Environmental changes gradually occur with time, and although genetic contribution of domesticated fish is increased in the nature due to the farmed escapees, natural selection does continuously occur and the new strains will have to replace the ones that no longer suitable to that environment. Both farmed escapees and wild fish living in the same environment will have to adapt with time. If we want to control the genetic integrity of fish in the wild, it is the environmental condition that has to be conserved. Otherwise we should let the natural evolutionary process occur (Rungruangsak-Torrissen 2002).

It is naive to think that genetically manipulated escapees such as triploid salmon will not impact genetic structure of wild population due to their infertility. We cannot expect the fish with gene manipulated to have exactly the same biochemical processes as the ordinary fish (Figure 12). Changes at molecular level will also affect changes at protein level, as well as fish behaviour. Generally, a change in environment (light regime) has a similar effect on both diploid and triploid Atlantic salmon (Figures 13 and 14). However, under a more favourable condition for growth, triploid escapees could compete with wild salmon on food availability as they require higher consumption rates for growth, unlike ordinary diploid salmon that could have better food utilisation at similar consumption rates (Figure 11). This may cause a higher survival rate in triploid escapees due to feeding hierarchy, and if they spawn, hatching success and survival rate of the offspring will be low due to low gamete quality in triploid fish. This could result in a smaller population of the new generation in that environment in the wild (Rungruangsak-Torrissen 2002).

Recruitment of salmon stocks through sea ranching is an important stock management programme. It is important that the cultured salmon used for stock enhancement should have low temperature experience during early life stage and be produced in the condition similar to the ecological condition where they will be released in order to increasing survival rate.

CONCLUSION

Studies of trypsin genotypes in Atlantic salmon have provided unique and excellent evidence on genetics of growth performance in connection with food utilization efficiency under different environments. Changes in environmental conditions are shown to influence gene expressions at molecular and protein levels, regardless of genetic expression of parents. This is very important for the discussion especially on the completion between escapees and wild salmon. Regardlessly, the evidence suggests that it is part of the genetic nature of salmon to adapt to a changing environment where new strains of salmon will fill the gap left by less adaptable strains (Rungruangsak-Torrissen 1999; 2002).

The knowledge on trypsin genotypes and food digestion and utilisation has also been used to study in other fish species (Rungruangsak Torrissen and Male 2000; Rungruangsak-Torrissen 2012) and integrated into fisheries research (Rungruangsak-Torrissen et al. 2012). Studies of trypsin specific activity, T/C ratio, and *in vitro* digestibility using dialyzed fish crude enzyme extracts, can provide practical informations on diet utilization and preferred food organisms. The studies also provide suggestions on fish production in nature when the environmental conditions (temperature, food availability) changes, whereas a reduction in fish size can be due to non-severe-changes in environmental temperature which interferes genetics in food utilization or due to higher food quality/availability that reduces impact on feeding hierarchy. Reduced fish size variations will reduce feeding hierarchy and increase survival rate, which can result in similar total fish production to normal condition or even higher production. Reduced fish size with high production has been observed in nature but ecologists and marine biologists could not explain this because they are not aware of genetic differences in food utilization caused by trypsin genotypes. This could be an important new strategy for future ecological studies for better understanding of living resources in natural ecosystems (see Rungruangsak-Torrissen et al. 2012). Variations in food digestion and utilization due to climate change will serve as tools for environmental impact assessment on fish production in nature. Trypsin genotypes have not yet been used, but this knowledge can be exploited and integrated into other types of research (see Chapter 7).

REFERENCES

Ablett, R.F., Sinnhuber, R.O., Holmes, R.M., Selivonchick, D.P., (1981). The effect of prolonged administration of bovine insulin in rainbow trout (*Salmo gairdneri* R.). *Gen. Comp. Endocrinol*. 43, 211–217.

Asgeirsson, B., Fox, J.W., Bjarnason, J.B., (1989). Purification and characterization of trypsin from the poikilotherm *Gadus morhua*. *Eur. J. Biochem.* 180, 85–94.

Bassompierre, M., Ostenfeld, T.H., McLean, E., Torrissen, K.R., (1998). *In vitro* protein digestion, and growth of Atlantic salmon with different trypsin isozymes. *Aquacult. Int*. 6, 47–56.

Blier, P.U., Lemieux, H., Devlin, R.H., (2002). Is the growth rate of fish set by digestive enzymes or metabolic capacity of tissues? Insight from transgenic coho salmon. *Aquaculture* 209, 379–384.

Coulson, R.A., Coulson, T.D., Herbert, J.D., Staton, M.A., (1987). Protein nutrition in the alligator. *Comp. Biochem. Physiol.* 87A, 449–459.

Craik, C.S.**,** Choo. Q.L., Swift, G.H., Quinto, C., McDonald, R.J., Rutter, W.J., (1984). Structure of two related rat pancreas trypsin genes. *J. Biol. Chem*. 259, 14255–14264.

Dahlmann, B., Jany, Kl.-D., (1975). A rapid and sensitive detection of proteolytic enzymes after electrophoresis. *J. Chromatogr*. 110, 174–177.

Fauconneau, B., Breque, J., Bielle, C., (1989). Influence of feeding on protein metabolism in Atlantic salmon (*Salmo salar*). *Aquaculture* 79, 29–36.

Fletcher, T.S., Alhadeff, M., Craik, **C.**S., Largman, C., (1987). Isolation and characterization of a cDNA encoding rat cationic trypsinogen. *Biochemistry* 26, 3081–3086.

Hedstrom, L., Farr-Jones, S., Kettner, C.A., Rutter, W.J., (1994b). Converting trypsin to chymotrypsin: Ground-state binding does not determine substrat specificity. *Biocheniistry* 33, 8764–8769.

Hedstrom, L., Perona, J.J., Rutter, W.J., (1994a). Converting Trypsin to chymotrypsin: Residue 172 is a substrate specificity determinant. *Biochemistry* 33, 8757–8763.

Hedstrom, L., Szilagyi, L., Rutter, W.J., (1992). Converting trypsin to chymotrypsin: The role of surface loops, *Science* 255, 1249–1253.

Hjelmeland, K., Raa, J., (1982). Characteristics of two trypsin type isoenzymes isolated from the artic fish capelin *(Mallotus villosus). Comp. Biochem. Physiol.* 71B, 557–562.

Holm, H., Hansen, L.P., Holst, J.C., Jacobsen, J.A., (2004). Atlantic salmon (*Salmo salar*). In H.R. Skjoldal (Ed.), *The Norwegian Sea Ecosystem* (pp. 315–356). Trondheim: Tapir Academic Press, Norway.

Ince, B.W., Thorpe, A., (1978). The effects of insulin on plasma amino acid levels in the Northern pike, *Esox lucius L. J. Fish Biol*. 12, 503–506.

Inui, Y., Arai, S., Yokote, M., (1975). Gluconeogenesis in the eel. VI. Effects of hepatectomy, alloxan and mammalian insulin on the behaviour of plasma amino acids. *Bull. Jap. Soc. Sci. Fish.* 41, 1105–1111.

Kiron, V., Fukuda, H., Takeuchi, T., Watanabe, T., (1993). Dietary protein related humoral immune response and disease resistance of rainbow trout, *Oncorhynchus mykiss*. In S.J. Kaushik, P. Luquet (Eds), *Fish Nutrition in Practice* (pp. 119–126). Paris: INRA Editions.

Love, R.M., (1980). The nature of fishes. In R.M. Love (Ed.), *The Chemical Biology of Fishes*, Vol. 2 (pp. 1–66). London: Academic Press, UK.

Machado, C.R., Garofalo, M.A.R., Roselino, J.E.C., Kettelhut, I.C., Migliorini, R.H., (1988). Effects of starvation, refeeding, and insulin on energy-linked metabolic processes in catfish (*Rhamdia hilarii*) adapted to a carbohydrate-rich diet. *Gen. Comp. Endocrinol*. 71, 429–437.

Male, R., Lorens, J.B., Smalås, A.O., Torrissen, K.R., (1995). Molecular cloning and characterization of anionic and cationic variants of trypsin from Atlantic salmon. *Eur. J. Biochem*. 232, 677–685.

Martinez, A., Olsen, R.L., Serra, J.L., (1988). Purification and characterization of two trypsin-like enzymes from the digestive tract of anchovy *Engraulis encracicholus, Comp. Biochem. Physiol.* 91B, 677–684.

Melingen, G.O., Stefansson, S.O., Berg, A., Wergeland, H.I., (1995). Changes in serum protein and IgM concentration during smolting and early postsmolt period in vaccinated and unvaccinated Atlantic salmon (*Salmo salar* L.). *Fish & Shellfish Immunol.* 5, 211–222.

Neji, H., Naimi, N., Lallier, R., De La Noüe, J., (1993). Relationships between feeding, hypoxia, digestibility and experimentally induced furunculosis in rainbow trout. In S.J. Kaushik, P. Luquet (Eds), *Fish Nutrition in Practice* (pp. 187–197). Paris: INRA Editions.

Perera, E., Moyano, F.J., Rodriguez-Viera, L., Cervantes, A., Martinez-Rodriguez, G., Mancera, J.M., (2010). *In vitro* digestion of protein sources by crude enzyme extracts of the spiny lobster *Panulirus argus* (Latreille, 1804) hepatopancreas with different trypsin isoenzyme patterns. *Aquaculture* 310, 178–185.

Rowen, L., Koop, B.F., Hood, L., (1996). The complete 685-kilobase DNA sequence of the human β T cell receptor locus. *Science* 272, 1755–1762.

Rungruangsak-Torrissen, K., (1999). *La den rømme. HAVBRUK — fiskeri og foredling*, Nr. 2/99, pp. 34–35. [in Norwegian].

Rungruangsak-Torrissen, K., (2002). Wild salmon should not be threatened by healthy and non-genetically manipulated escapees. *ICES CM 2002 Documents, ICES Conference, CM 2002/T:07,* 16 pp., October 1–5, Copenhagen, Denmark.

Rungruangsak-Torrissen, K., (2012). Trypsin and its implementations for growth, maturation, and dietary quality assessment. In K. Weaver, C. Kelley (Eds.), *Trypsin*: Structure, Biosynthesis and Functions (pp. 1–59). New York: Nova Science Publishers, Inc., USA. *https://www.novapublishers.com/catalog/product_info.php?products_id=38114*

Rungruangsak-Torrissen, K., Carter, C.G., Sundby, A., Berg, A., Houlihan, D.F., (1999a). Maintenance ration, protein synthesis capacity, plasma insulin and growth of Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes. *Fish Physiol. Biochem*. 21, 223–233.

Rungruangsak-Torrissen, K., Fosseidengen, J. E., (2007). Effect of artificial feeding on digestive efficiency, growth and qualities of muscle and oocyte of maturing Atlantic mackerel (*Scomber scombrus* L.). *J. Food Biochem.* 31, 726–747.

Rungruangsak Torrissen, K., Male, R., (2000). Trypsin Isozymes: Development, Digestion and Structure. In N.F. Haard, B.K. Simpson (Eds.), *Seafood Enzymes*: Utilization and influence on postharvest seafood quality (pp. 215–269). New York: Marcel Dekker, Inc., USA.

Rungruangsak-Torrissen, K., Moss, R., Andresen, L.H., Berg, A., Waagbø, R., (2006). Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (*Salmo salar* L.). *Fish Physiol. Biochem.* 32, 7–23.

Rungruangsak-Torrissen, K., Pringle, G.M., Moss, R., Houlihan, D.F., (1998). Effects of varying rearing temperatures on expression of different trypsin isozymes, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar* L.). *Fish Physiol. Biochem*. 19, 247–255.

Rungruangsak-Torrissen, K., Stensholt, B.K., (2001). Spatial distribution of Atlantic salmon post-smolts: Association between genetic differences in trypsin isozymes and environmental variables. In G.H. Kruse, N. Bez, A. Booth, M.W. Dorn, S. Hills, R.N. Lipcius, D. Pelletier, C. Roy, S.J. Smith, D. Witherell (Eds.), *Spatial Processes and*

Management of Marine Populations (pp. 415–429). Fairbanks: University of Alaska Sea Grant, AK-SG-01-02.

Rungruangsak-Torrissen, K., Stien, L.H., Daae, B.S., Vågseth, T., Thorsheim, G.B., Tobin, D., Ritola, O., (2009b). Different dietary levels of protein to lipid ratio affected digestive efficiency, skeletal growth, and muscle protein in rainbow trout families. *Scholarly Research Exchange*, vol. 2009, Article ID 709529, doi:10.3814/2009/709529. *http://www.hindawi.com/archive//2009/709529/abs/*

Rungruangsak-Torrissen, K., Sundby, A., (2000). Protease activities, plasma free amino acids and insulin at different ages of Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes. *Fish Physiol. Biochem*. 22, 337–347.

Rungruangsak-Torrissen, K., Sunde, J., Berg, A.E., Nordgarden, U., Fjelldal, P.G., Oppedal, F., (2009a). Digestive efficiency, free amino acid pools and quality of growth performance in Atlantic salmon *(Salmo salar* L.) affected by light regimes and vaccine types. *Fish Physiol. Biochem.* 35, 255–272.

Rungruangsak-Torrissen, K., Thongprajukaew, K., Sansuwan, K., Thapthimdaeng, P., Kovitvadhi, U., Seetaha, S., Choowongkomon, K., Beck, I.M., Arnøy, O.O., (2012). Ecological effects on food utilization, trypsin isozymes, and performance qualities of growth and maturation in Northeast Arctic cod (*Gadus morhua* L.). *The Open Fish Science Journal* 5, 44–56. *http://benthamscience.com/open/openaccess.php?tofishsj/articles/V005/44TOFISHSJ.htm*

Rungruangsak-Torrissen, K., Wergeland, H.I., Glette, J., Waagbø, R., (1999b). Disease resistance and immune parameters in Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes. *Fish and Shellfish Immunol*. 9, 557–568.

Røed, K.H., Fjalestad, K.T., Strømsheim, A., (1993). Genetic variation in lysozyme activity and spontaneous haemolytic activity in Atlantic salmon (*Salmo salar*). *Aquaculture* 114, 19–31.

Simpson, B.K., Haard, N. F., (1984). Purification and characterization of trypsin from the Greenland cod *(Cadus ogac.).* 1. Kinetic and thermodynamic characteristics. *Can. J. Biochem. Cell Biol.* 62, 894–900.

Smalås, A.O., Heimstad, E.S., Hordvik, A., Willassen, N.P., Male, R., (1994). Cold-adaption of enzymes: Structural comparison between salmon and bovine trypsins. *Proteins* 20, 149–166.

Sundby, A., Eliassen, K., Refstie, T., Plisetskaya, E.M., (1991). Plasma levels of insulin, glucagon and glucagon-like peptide in salmonids of different weights. *Fish Physiol. Biochem*. 9, 223–230.

Sunde, J., Eiane, S.A., Rustad, A., Jensen, H.B., Opstvedt, J., Nygård, E., Venturini, G., Rungruangsak-Torrissen, K., (2004). Effect of fish feed processing conditions on digestive protease activities, free amino acid pools, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar* L.). *Aquacult. Nutr*. 10, 261–277.

Sunde, J., Taranger, G.L., Rungruangsak-Torrissen, K., (2001). Digestive protease activities and free amino acids in white muscle as indicators for feed conversion efficiency and growth rate in Atlantic salmon (*Salmo salar* L.). *Fish Physiol. Biochem*. 25, 335–345.

Taran, L.D., Smovdyr, I.N., (1992). Comparative kinetic investigations of the primary specificity of bovine and salmon trypsins. *Biokhimiya* 57, 55–60.

Torrissen, K.R., (1984). Characterization of proteases in the digestive tract of Atlantic salmon (*Salmo salar*) in comparison with rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol*. 77B, 669–674.

Torrissen, K.R., (1987). Genetic variation of trypsin-like isozymes correlated to fish size of Atlantic salmon (*Salmo salar*). *Aquaculture* 62, 1–10.

Torrissen, K.R., (1991). Genetic variation in growth rate of Atlantic salmon with different trypsin-like isozyme patterns. *Aquaculture* 93, 299–312.

Torrissen, K.R., Barnung, T.N., (1991). Genetic difference in trypsin-like isozyme pattern between two strains of Arctic charr (*Salvelinus alpinus*). *Aquaculture* 96, 227–231.

Torrissen, K.R., Lied, E., Espe, M., (1994). Differences in digestion and absorption of dietary protein in Atlantic salmon (*Salmo salar*) with genetically different trypsin isozymes. *J. Fish Biol*. 45, 1087–1104.

Torrissen, K.R., Lied, E., Espe, M., (1995). Differences in utilization of dietary proteins with varying degrees of partial pre-hydrolysis in Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes. In J. Svasti, V. Rimphanitchayakit, A. Tassanakajorn, P. Pongsawasdi, B. Sonthayanon, K. Packdibamrung, S. Soontaros, T. Limpaseni, P. Wilairat, J. Boonjawat, S. Kamolsiripichaiporn (Eds.), Biopolymers and Bioproducts: Structure, Function and Applications (pp. 432–442). *Proceedings of the 11th FAOBMB Symposium* (IUBMB Symposium No.239). Bangkok: Samakkhisan Public Company Limited, ISBN 974-632-655-4.

Torrissen, K.R., Male, R., Nævdal, G., (1993). Trypsin isozymes in Atlantic salmon, *Salmo salar* L.: studies of heredity, egg quality and effect on growth of three different populations. *Aquacult. Fish. Manage*. 24, 407–415.

Torrissen, K.R., Shearer, K.D., (1992). Protein digestion, growth and food conversion in Atlantic salmon and Arctic charr with different trypsin-like isozyme patterns. *J. Fish Biol*. 41, 409–415.

Torrissen, K.R., Torrissen, O.J., (1985). Protease activities and carotenoid levels during the sexual maturation of Atlantic salmon (*Salmo salar*). *Aquaculture* 50, 113–122.

Waagbø, R., (1994). The impact of nutritional factors on the immune system in Atlantic salmon, *Salmo salar* L.: a review. *Aquacult. Fish. Manage.* 25, 175–197.