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Salmon Aquaculture, Enhancement, and Ranching: are they a Threat to Wild Salmonid Stock

Wild salmon should not be threatened by healthy and non-genetically manipulated escapees

Krisna Rungruangsak-Torrissen

Institute of Marine Research, Department of Aquaculture,
Matre Aquaculture Research Station, N-5984 Matredal, Norway. [e-mail: Krisnart@imr.no]

Abstract

Cultured salmon as escapees should never threaten salmon stocks in the wild as long as they are healthy and are not genetically manipulated. By studying a key digestive protease, trypsin, which is sensitive to environmental changes and influences on food utilisation and growth during the whole life cycle of Atlantic salmon, it is indicated that changes in the phenotypic expression of trypsin can be induced by temperature during egg incubation and the start-feeding period of the alevins. In addition, Atlantic salmon with the same trypsin phenotype showed different feed conversion efficiency and growth rate at different temperatures. Trypsin genes seem to be stable, and although the pattern of expressed genes varies extensively, the expression of trypsinogen mRNA is quantitatively similar between individual salmon in line with the observation that the total trypsin specific activity was similar between the fish with different trypsin phenotypes. However the luminal secretion of the active enzyme, and probably the relative amounts of trypsin isozymes, could be modified by water temperature and food quality. These results indicate that changes in the environmental condition can influence gene expressions of the fish at DNA, RNA and protein levels, regardless of genetic expression of parents. This means that whether the escapees or wild fish is the spawning population, an incidence of the offsprings to have their gene expressions adapted to that environment will be similar. It is the environmental condition that has to be conserved in order to control the genetic structure of animals in the wild.

It is naive to think that genetically manipulated escapees such as triploid salmon will not have any impact on wild population, as they are not fertile. Under a more favourable condition for growth, triploid escapees could compete with wild fish on food availability as they have higher consumption rates for higher growth rates, unlike ordinary diploid salmon that could have better food utilisation at a similar consumption rate. This may cause a higher survival rate in triploid escapees and if they spawn, hatching success and survival rate of the offsprings will be low due to low gamete quality for reproduction in triploid fish. This could result in a smaller population of the new generation in that environment in the wild.

Keywords: Atlantic salmon, escapees, trypsin phenotype, trypsin specific activity, activity ratio of trypsin to chymotrypsin, triploid, temperature

Introduction

The escape of farmed salmon has created a lot of debate on possibilities of having effect on the decline of wild salmon stocks (Carr *et al*., 1997; Beamish *et al*., 1997; Noakes *et al*., 2000; Soto *et al*., 2001), un-successful in increasing salmon abundance through salmon release as adults (Berejikian *et al*., 2001), and irreversible changes in genetic structure of wild populations (Lura and Saegrov, 1991; Mork, 1991; Crozier, 1993; McGinnity *et al*., 1997; Youngson *et al*., 2001). Most works on wild and farmed salmonids showed differences in behavior and growth between them, and suggested a possibility of threaten native populations by escaped farmed conspecifics through competition and disruption of local adaptation (Ruzzante, 1994; Johnsson *et al*., 1996; Einum and Fleming, 1997; Fleming *et al*., 2000; Petersson and Jarvi, 2000; Reinhardt *et al*., 2001). Suggestions of using triploids to reduce the ecological impact of escaped farmed fish were reported (Cotter *et al*., 2000; Youngson *et al*., 2001). Without any scientific evidence to directly prove these points, these debates cannot be ended.

This presentation is a synthesis of different published works including unpublished data, trying to make a benefit of scientific knowledge in aquaculture to explain some possible natural/logical interactions in the wild between farmed salmon as escapees and wild conspecific populations in a different point of view. The work is based on the knowledge on food utilization and growth in Atlantic salmon cultured in different environmental conditions in connection with different expressions of trypsin (including chymotrypsin) in the digestive system at both molecular and protein levels, which were intensively reviewed in Rungruangsak Torrissen and Male (2000).

Interactions between environments and trypsin expressions

Trypsin is a proteolytic enzyme in the digestive system, found in different isoforms. Besides genetic variation (Torrissen, 1987; Torrissen, 1991; Torrissen *et al.*, 1994), differences in trypsin expression are influenced by external factors such as temperature (Rungruangsak-Torrissen *et al.*, 1998), light (Cuvier-Peres *et al*., 2001; Sunde *et al*., in press; Rungruangsak-Torrissen, submitted revised version), pH (Woo *et al.*, 1997), endocrine peptides such as cholecystokinin (Einarsson *et al*., 1997), growth hormone (Lemieux *et al*., 1999), and dietary quality (Haard *et al.*, 1996; El-Saidy *et al.*, 2000). Trypsin expression is known to be a very important rate-limiting factor for feed utilisation and growth (Torrissen and Shearer, 1992; Rungruangsak-Torrissen *et al*., 1998; Lemieux *et al*., 1999). Atlantic salmon with different trypsin phenotypes showed different maintenance ration and capacity for protein synthesis (Rungruangsak-Torrissen *et al*., 1999). If standardizing fish crude enzyme extracts for *in vitro* digestibility study with regards to trypsin activity, the *in vitro* digestibility values could be comparable not only within the same species but also between different species (Rungruangsak-Torrissen *et al*., 2002). Trypsin specific activity and the activity ratio of trypsin to chymotrypsin (T/C ratio) are related with specific growth rate and feed conversion efficiency, and these factors could possibly be applied for studies in the natural ecosystems for comparison and prediction of potential differences in food utilization and growth without necessity to know consumption rate (Sunde *et al*., submitted revised version).

Rungruangsak-Torrissen *et al*. (1998) demonstrated that trypsin phenotype of individuals is established during the early life stage, which is influenced by water temperature during egg incubation and start-feeding period of alevins. The experiment was performed from mixed fertilised eggs, obtained from 17 females and 9 males,

randomly divided into two groups for incubation in cold (5.9±1.9 ^{o}C) and warm (9.6±1.2 ^{o}C) water, and the alevins from each incubation temperature were divided into two sub-groups for start-feeding under temperature controls during February to June 1994 in cold (5.6±1.3 ^{o}C) and warm (12.2±0.5 ^{o}C) water. After that the fingerlings were cultured in natural water temperatures of around 10^{o}C from July to November 1994 and around 6^{o}C from December 1994 to January 1995. The frequency distributions of different trypsin isozyme patterns (pattern 1: TRP-2*100/100, pattern 2: TRP-2*100/92, and pattern 2′: TRP-2*92/92) were studied for the expressions when the fish had been in warm water condition in November 1994 (10.2±1.5^{o}C) and also in cold water condition in January 1995 (6.1±0.5^{o}C). Within the same sub-group, there was no difference in the frequency distribution of different trypsin phenotypes under warm and cold water conditions. This means that the expressions of trypsin phenotypes of individuals had already been established. On the other hand, the frequency distributions between the different trypsin phenotypes were varied between the four groups of Atlantic salmon parr (Table 1). The effects of warm hatching temperature on the increased expression of trypsin isozyme TRP-2*100, and of cold hatching temperature on the increased expression of the variant TRP-2*92 were observed as the ratios between patterns 2 and 2′ were changed from 0.68:0.32 in warm hatching to 0.55:0.45 in cold hatching, regardless of start-feeding temperature ($p<0.01$ by Chi-square and Fisher's test). In contrast to hatching, warm start-feeding temperature increased the expression of trypsin variant TRP-2*92 as the ratios between fish without (pattern 1) and with (patterns 2+2′) the variant were changed from 0.30:0.65 in cold start-feeding to 0.17:0.78 in warm start-feeding, regardless of hatching temperature ($p<0.0001$). The isozyme TRP-2*100 was found to be functionally effective at temperature > 8^{o}C, whilst the variant TRP-2*92 at ≤ 8^{o}C and especially below 6^{o}C (Rungruangsak-Torrissen *et al*., 1998). We do not know why warm start-feeding temperature induced the expression of the cold temperature functioning isozyme TRP-2*92. Rungruangsak Torrissen and Male (2000) further demonstrated that the development of trypsin in the pyloric caeca was faster with higher specific activity when the alevins were start-fed at warm than at cold temperature, and the faster growth fish had higher trypsin specific activity.

Study 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 1995) indicated that these enzyme values varied according to trypsin phenotypes, past temperature experience during early feeding, and present environmental temperature (Table 1). The fish possessing trypsin phenotype TRP-2*92/92 (pattern 2′) with the trypsin variant effectively functioning at temperature < 6^{o}C showed a relatively higher value of either trypsin specific activity (80.1±5.7 versus 68.5±3.7 μmol *p*-nitroaniline produced h^{-1} mg protein^{-1}) or both trypsin specific activity (73.4±5.3 versus 72.3±3.6 μmol p-nitroaniline produced h^{-1} mg protein^{-1}) and T/C ratio (11.7±2.1 versus 9.5±0.9) than the pattern 1 fish (TRP-2*100/100) without the variant, if both phenotypes had cold start-feeding temperature experience. These enzyme values were vice versa (pattern 1 > pattern 2′) 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 phenotypes within the same temperature control group, except for the group of warm hatching and warm start-feeding temperatures that the fish lacking the cold temperature variant (pattern 1: 46.3±4.5 g) were significantly smaller ($p<0.05$) than the fish carrying the variant (pattern 2: 65.7±3.7 g, pattern 2′: 61.2±4.8 g). Although the pattern 1 fish having warm start-feeding temperature experience seemed to be smaller than the pattern 2′ fish during the first winter due to lacking cold temperature

Table 1. Number of sampled fish, frequency distribution of each trypsin phenotype, protease specific activities of trypsin and chymotrypsin (μmol *p*-nitroaniline produced h^{-1} mg $protein^{-1}$) and activity ratio of trypsin to chymotrypsin (T/C ratio) during the first winter (November 1994–January 1995) of Norwegian Atlantic salmon parr, hatched and start-fed at different temperatures (February-June 1994). The values with different superscripts or with asterisk (*) are significantly different ($p<0.05$ by ANOVA). For the pattern ratios, within the same column, the ratios with different superscripts are significantly different ($p<0.01$ by Chi-square and Fisher's test). Data were adapted from Rungruangsak-Torrissen *et al*. (1998) where the details of different trypsin phenotypes and experimental methods were described. The methods for determination of trypsin and chymotrypsin are described in Rungruangsak-Torrissen and Sundby (2000).

Temperature	Parameter	Trypsin isozyme pattern				Pattern ratio	
(°C)	(mean±sem)	1	2	2′	Others	1 : 2+2′	2 : 2′
Hatching 5.9±1.9	Frequency	74 (0.27)	98 (0.36)	81 (0.30)	19 (0.07)	[a]0.27:0.66	[a]0.56:0.44
	Weight (g)	14.6±0.5	15.6±0.4	15.4±0.5			
Start-feeding 5.6±1.3	Trypsin[#]	68.5±3.7	72.4±4.2	80.1±5.7			
	Chymotrypsin[#]	[a]7.9±0.6	[a]7.5±0.5	[b]9.6±0.7			
	T/C ratio[#]	9.9±1.0	10.7±0.9	9.3±1.1			
Hatching 5.9±1.9	Frequency	40 (0.17)	101 (0.43)	84 (0.36)	10 (0.04)	[b]0.17:0.79	[a]0.54:0.46
	Weight (g)	34.6±2.0	33.0±1.0	34.8±1.4			
Start-feeding 12.2±0.5	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	Frequency	81 (0.32)	111 (0.44)	50 (0.20)	11 (0.04)	[a]0.32:0.64	[b]0.70:0.30
	Weight (g)	17.5±0.7	20.0±0.9	16.1±1.0			
Start-feeding 5.6±1.3	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	Frequency	49 (0.17)	146 (0.52)	69 (0.25)	17 (0.06)	[b]0.17:0.77	[b]0.68:0.32
	Weight (g)	[a]46.3±4.5	[b]65.7±3.7	[b]61.2±4.8			
Start-feeding 12.2±0.5	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			

[#] Unpublished data measured in January 1995.

functioning isozyme TRP-2*92, they would grow faster later when water temperature increased as they had somewhat higher trypsin specific activity and T/C ratio according to Rungruangsak-Torrissen (2001). Winter temperature had a higher adverse effect on pattern 1 fish if they had no earlier cold temperature experience. The pattern 2 salmon (TRP-2*100/92) showed better performance than the other phenotypes at varying temperature control conditions (Table 1). Interestingly, trypsin specific activity (78.8±2.1 versus 72.7±1.8 μmol *p*-nitroaniline produced h^{-1} mg $protein^{-1}$, $p<0.03$) and T/C ratio (18.7±0.7 versus 9.9±0.5, $p<0.0001$) were significantly higher, while chymotrypsin specific activity (4.5±0.1 versus 8.6±0.3 μmol *p*-nitroaniline produced h^{-1} mg $protein^{-1}$, $p<0.0001$) was significantly lower, in higher growth salmon (47.9±1.9 g versus 16.6±0.3 g) having warm start-feeding temperature experience than slower growth fish having cold start-feeding temperature experience, regardless of hatching temperatures and trypsin phenotypes. This is in accordance with the proposition described by Rungruangsak-Torrissen (2001) that trypsin is the key protease under condition favouring growth, while chymotrypsin plays a major role when growth opportunity is interrupted or limited.

Table 2. Weight, specific growth rate (SGR) and feed conversion efficiency (FCE, wet weight gain per dry weight of feed consumed) of Scottish Atlantic salmon parr of different trypsin phenotypes, cultured at different temperature regimes for 121 days during April to August 1994. The values with different superscripts or with asterisk (*) are significantly different ($p<0.05$ by ANOVA). Data were adapted from Rungruangsak-Torrissen *et al*. (1998).

(mean±sem)	3.8–14.4 °C	7.3–17.4 °C	11.9–19.9 °C
Pattern 1: TRP-2*100/100			
Number (n)	217	74	70
Initial weight (g)	4.81±0.13	5.56±0.21	5.68±0.20
Final weight (g)	[a]9.87±0.23	[a]10.55±0.44	[b]13.64±0.42
SGR (% day^{-1})	[a]0.64±0.01	[a]0.65±0.02	[b]0.93±0.02
FCE	[a]0.95±0.02	[b]0.73±0.03	[b]0.81±0.02
Pattern 2: TRP-2*100/92			
Number (n)	50	31	22
Initial weight (g)	5.91±0.31	[*]6.21±0.22	[*]5.29±0.30
Final weight (g)	10.64±0.50	12.06±0.55	13.05±0.55
SGR (% day^{-1})	[a]0.59±0.03	[a]0.67±0.04	[b]0.97±0.05
FCE	[a]0.92±0.05	[b]0.75±0.04	[a]0.91±0.06
Pattern 2′: TRP-2*92/92			
Number (n)	21	10	1
Initial weight (g)	5.64±0.44	5.84±0.58	3.05
Final weight (g)	10.30±0.76	10.56±1.57	7.51
SGR (% day^{-1})	0.59±0.04	0.55±0.09	0.95
FCE	[*]0.90±0.06	[*]0.56±0.09	0.69

In addition, Rungruangsak-Torrissen *et al*. (1998) demonstrated that Atlantic salmon of the same trypsin phenotype when reared at different temperature regimes showed different feed conversion efficiency (FCE) and specific growth rate according to trypsin phenotype of the individuals (Table 2). The fish with TRP-2*92/92 phenotype grew relatively faster in colder (0.59±0.04 % day^{-1} at 3.8–14.4 °C) than in warmer (0.55±0.09 % day^{-1} at 7.3–17.4 °C) temperature regime, while the fish with phenotype TRP-2*100/100 performed better growth at higher temperature regime (0.64±0.01 % day^{-1} at 3.8–14.4 °C, 0.65±0.02 % day^{-1} at 7.3–17.4 °C, 0.93±0.02 % day^{-1} at 11.9–19.9 °C). The heterozygotes pattern 2 (TRP-2*100/92) showed a better growth performance than the other phenotypes in all temperature regimes studied. Atlantic salmon seemed to have a higher FCE at a lower water temperature regime, regardless of trypsin phenotypes (Table 2).

There is a significant correlation between trypsin specific activity and the T/C ratio value on individual basis (Fig. 1). Although chymotrypsin, unlike trypsin, is not always associated with growth rate and feed conversion efficiency (Lemieux *et al.*, 1999; Sunde *et al.*, submitted revised version), it is also important as it affects the T/C ratio value (Rungruangsak Torrissen and Male, 2000; Rungruangsak-Torrissen and Sundby, 2000), which correlates with specific growth rate (Sunde *et al.*, submitted revised version). At the same restricted amount of feeding (based on 0.5% of body weight of the fish reared at 6°C) to achieve a similar growth rate between the fish reared at 6°C and 12°C, trypsin specific activity seemed to be higher at low (6°C: 24–138 μmol *p*-nitroaniline h^{-1} mg protein^{-1}) than at high (12°C: 12.5–124 μmol *p*-nitroaniline h^{-1} mg protein^{-1}) temperature, in contrast to chymotrypsin specific activity (6°C: 1.6–12.1 μmol *p*-nitroaniline h^{-1} mg protein^{-1}; 12°C: 2.9–12.4 μmol *p*-nitroaniline h^{-1} mg protein^{-1}). This

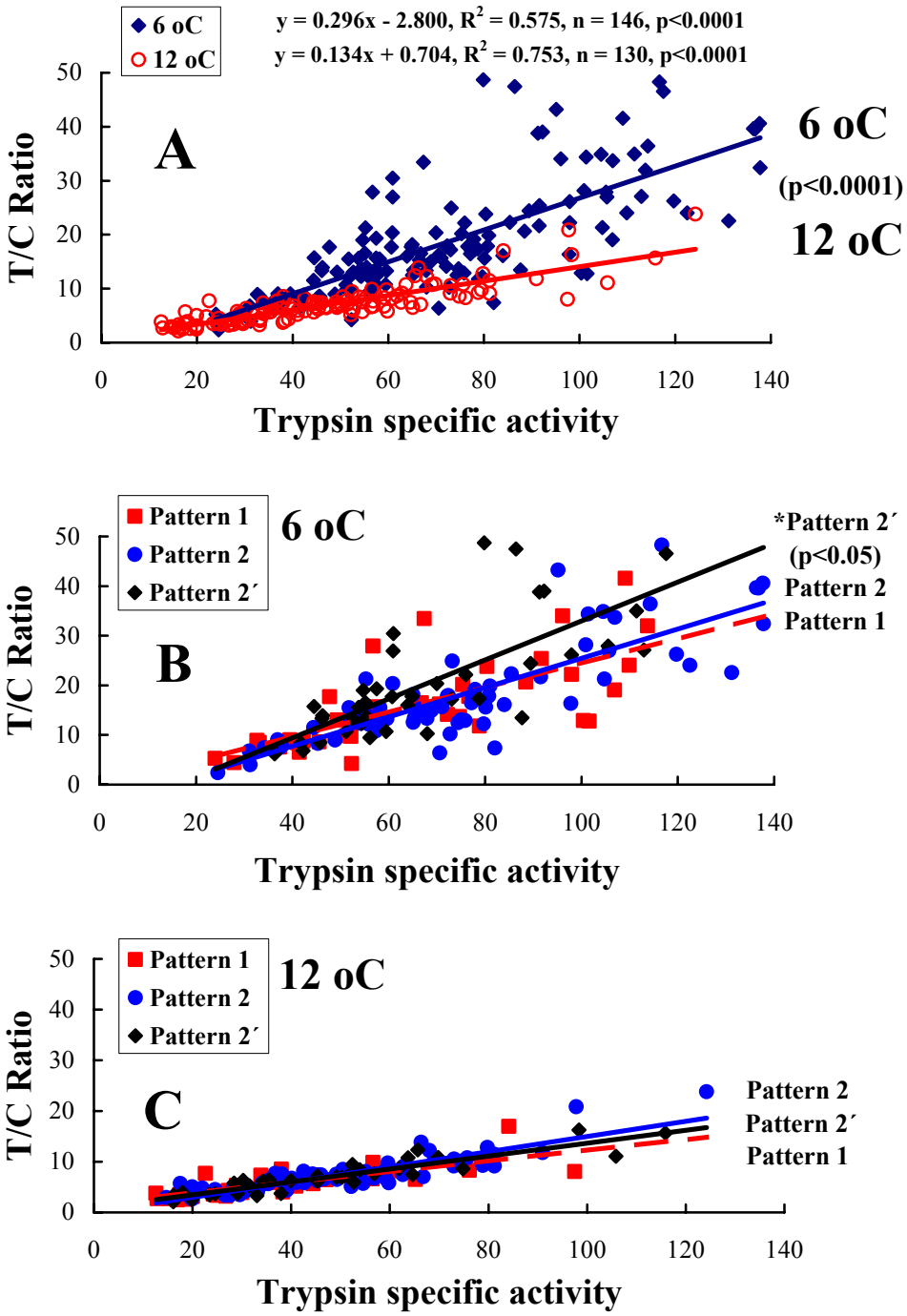

Figure 1. Relationships between trypsin specific activity and the protease activity ratio of trypsin to chymotrypsin (T/C ratio) in Atlantic salmon (10–70 g) at different rearing temperatures (6°C and 12°C), regardless of trypsin phenotypes **[A]**, and according to trypsin phenotypes at 6°C **[B]** and 12°C **[C]**. The fish were divided into 4 groups according to their weights (10–19 g, 20–35 g, 36–49 g, and 50–70 g) and reared for 3 months. The same restricted amount of feed, based on 0.5% of body weight of the fish reared at 6°C, was used to feed the fish at both temperatures to achieve a similar growth rate. Trypsin phenotypes were identified at the end when pyloric caeca samples were collected. The fish of pattern 2´ possess a trypsin variant effectively functioning at ≤ 8°C, pattern 1 fish do not possess that trypsin variant, and pattern 2 fish are heterozygotes of pattern 1 and pattern 2´. Descriptions of different trypsin patterns were reported in Rungruangsak-Torrissen *et al.* (1998), and Rungruangsak Torrissen and Male (2000). The enzyme specific activities are expressed as µmol *p*-nitroaniline h^{-1} mg $protein^{-1}$ in the whole pyloric caeca with food content. (Unpublished data).

has made the T/C ratio values higher when the fish were reared at low than at high temperature, based on the same specific activity of trypsin (Fig. 1A). This could be due to the very restricted ration causing a limitation in growth rates of the fish reared at 12°C. The results indicated a lower T/C ratio value due to a higher secretion of chymotrypsin in fish having a limitation/interruption in growth rate, as also shown in Table 1 and as described by Rungruangsak-Torrissen (2001). At 6°C (Fig. 1B), the T/C ratio values were higher in the pattern 2´ fish, compared to the fish of pattern 2 and pattern 1, corresponding with the previous observations that the fish with pattern 2´ and pattern 2 had higher growth rates than the pattern 1 fish at rearing temperature of ≤ 8°C, especially below 6°C (Torrissen *et al.*, 1993; Rungruangsak-Torrissen *et al.*, 1998; see also Table 2). On the other hand, the T/C ratio values were similar between different trypsin phenotypes at 12°C when growth was limited due to the very restricted ration (Fig. 1C). These phenomena of temperature effects on the secretions of the trypsin and chymotrypsin had been previously observed (Rungruangsak-Torrissen and Sundby, 2000). Atlantic salmon possessing different trypsin phenotypes expressed different trypsin specific activity (Table 1; Rungruangsak Torrissen and Male, 2000) and T/C ratio values (Table 1, Fig. 1B), and exhibited different growth performance at different temperatures (Table 2; Rungruangsak-Torrissen *et al.*, 1998) and feeding rates (Rungruangsak-Torrissen *et al.*, 1999). This shows that food utilisation is a very sensitive process in fish. Higher variations in growth rates found at different environmental conditions are usually due to genetic differences in food utilisation of the individuals.

Rungruangsak-Torrissen and Stensholt (2001) reported a similar performance in Atlantic salmon post-smolts in the natural marine ecosystem, off the Hebrides area (56–60°N, 11°W–2°E) and in the Norwegian Sea (62–72°N, 16°E–7°W) (Fig. 2). Post-smolts, caught as bycatch from a pelagic trawl survey in the northeastern Atlantic Ocean during summer 1995, revealed their differences in growth and temperature preference according to their trypsin phenotypes. During sea migration, food availability is very important for survival. Off the Hebrides area where the ambient temperature was around 10.2°C with relatively high ichthyoplankton indices (as determined acoustically), the fish of pattern 1 seemed to grow faster than the other phenotypes as the mean weight of pattern 1: pattern2: pattern 2´ was 64 g: 62 g: 58 g. Contrastingly, in the Norwegian Sea under lower ichthyoplankton densities, post-smolts were distributed and grew differently according to temperature preference of each trypsin phenotype, showing distribution of pattern 1: pattern 2: pattern 2´ at ambient temperature of 9.3°C: 8.7°C: 7.7°C with average weight of 133 g: 137 g: 234 g. Growth of Atlantic salmon post-smolts in the ocean seemed to be affected by food utilisation at different ambient temperatures and to be dependent on the trypsin phenotype possessed by individuals.

Several trypsin genes from Atlantic salmon have been characterised and the expression of trypsinogen mRNA seemed to be quantitatively similar between individual salmon by Northern hybridisations using RNA from individual fish (Male *et al*., 1995). This in line with the observation demonstrated by Rungruangsak Torrissen and Male (2000) that total trypsin specific activity in the pyloric caeca (in both tissues and lumen) was similar between salmon with different trypsin phenotypes (Table 3). However, the level of trypsin in the pyloric caecal tissues was significantly higher in the salmon lacking (52.05±3.15 μmol p-nitroaniline produced h^{-1} mg $protein^{-1}$) than the salmon carrying (38.10±3.77 μmol p-nitroaniline produced h^{-1} mg $protein^{-1}$) the variant TRP-2*92 ($p<0.02$), suggesting a lower level of trypsin secreted into the lumen in salmon without than with the variant. No differences in chymotrypsin levels were observed between different trypsin phenotypes. Total enzyme specific activities in the whole caeca were not directly comparable to those in the caecal tissues due to high protein in the food content.

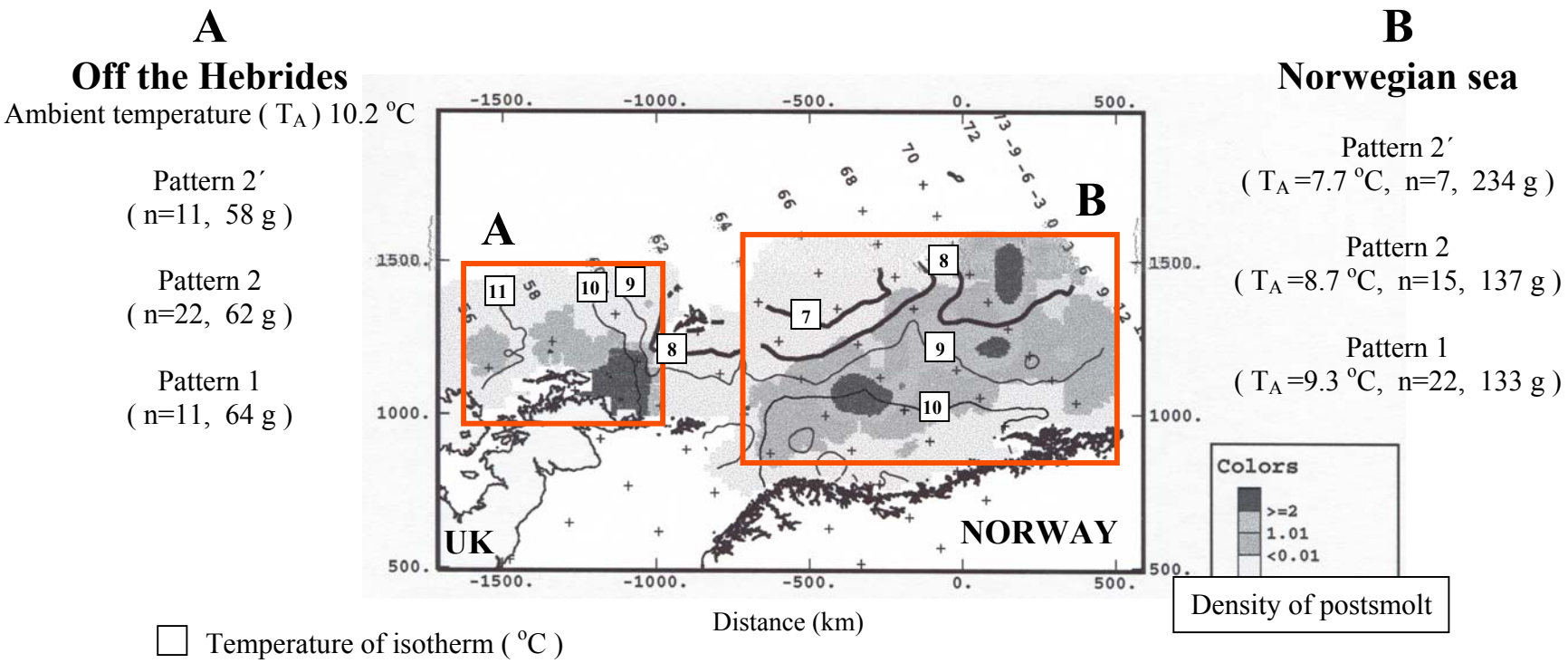

Figure 2. Spatial distribution and average body weight of Atlantic salmon post-smolts with pattern 1 (TRP-2*100/100), pattern 2 (TRP-2*100/92), and pattern 2´ (TRP-2*92/92) at different ambient temperatures (T_A) off the Hebrides area (June 1995) and in the Norwegian Sea (July 1995). Temperature of each isotherm is labeled. The T_A value (°C) was calculated and defined as average temperature weighted by fish density for each trypsin phenotype in each area (see Rungruangsak-Torrissen and Stensholt, 2001). Numbers of post-smolts caught per nautical mile are indicated by gray shades. The distance in km is indicated on the frame. Latitude and longitude are indicated in the picture. Data were adapted from Rungruangsak-Torrissen and Stensholt (2001).

Table 3. Protease specific activities of trypsin and chymotrypsin (μmol *p*-nitroaniline produced h^{-1} mg $protein^{-1}$) at 5–7 h post-feeding and the activity ratio of trypsin to chymotrypsin (T/C ratio), in the pyloric caecal tissues (empty caeca without luminal content) and in the whole pyloric caeca (with luminal content), of Atlantic salmon with (n=19) and without (n=17) trypsin variant TRP-2*92. The values with different superscripts are significantly different ($p<0.02$). Data were adapted from Rungruangsak Torrissen and Male (2000).

Protease specific activity (mean±sem)	Pyloric caecal tissues		Whole pyloric caeca	
	Without	With	Without	With
Trypsin (T)	[a]52.05±3.15	[b]38.10±3.77	[b]36.23±5.02	41.39±4.61
Chymotrypsin (C)	[a]28.48±2.37	[a]27.88±2.39	[b]64.41±5.37	[b]68.77±5.15
T/C ratio	[a]1.95±0.15	[a]1.53±0.16	[b]0.62±0.11	[b]0.64±0.09

Besides genetic factor, the luminal secretion of the active trypsin, and probably the relative amounts of the trypsin isozymes, could be modified in individual fish according to different factors such as water temperature and food quality, especially at the very early life stage (Rungruangsak Torrissen and Male, 2000; Rungruangsak-Torrissen, 2001; present paper).

All the results above indicated that changes in the environmental condition can influence gene expressions of the fish at DNA, RNA and protein levels, regardless of the genetic expression of parents.

Feed utilisation in diploid and triploid Atlantic salmon

Sunde *et al*. (submitted revised version) has demonstrated differences in feed utilisation and growth performance between diploid and triploid Atlantic salmon of about 1 kg in weight, reared in different light regimes (Fig. 3). Correlations are high ($r=0.98$, $p<0.02$) between specific growth rate (SGR), feed conversion efficiency (FCE), specific consumption rate (SCR) and the T/C ratio. Continuous light regime (LL) stimulated growth by increasing these parameters. The difference between diploid and triploid salmon in increasing growth rate was that diploid fish could improve their SGR and FCE through a similar SCR (see the values of 2N-SNP and 2N-LL groups in Fig. 3), whilst triploid fish improved their SGR and FCE through increased SCR (see the values of 3N-SNP and 3N-LL groups in Fig. 3). These results indicated that, under a living condition favouring growth, triploid salmon could compete with diploid fish on food availability due to their higher consumption rates. Under the condition studied on simulated natural light period (SNP), triploid salmon had a lower feed utilisation and growth performance than the diploid salmon, showing a relatively lower FCE, SGR, and SCR as well as the T/C ratio value (Fig. 3).

Trypsin specific activity and T/C ratio value are important factors for comparing food utilisation and potential growth rate between different fish groups. In gene-manipulated triploid Atlantic salmon where chymotrypsin could not be directly comparable with diploid fish (Fig. 3), the T/C ratio value showed a correlation with specific growth rate as trypsin specific activity on individual basis (n=80, Sunde *et al.*, submitted revised version) as well as on group basis (n=4, Fig. 3), regardless of ploidy and light regime. A high sensitivity of the T/C ratio value for digestive ability was observed as the correlations between T/C ratio value, SGR, FCE and SCR were highly

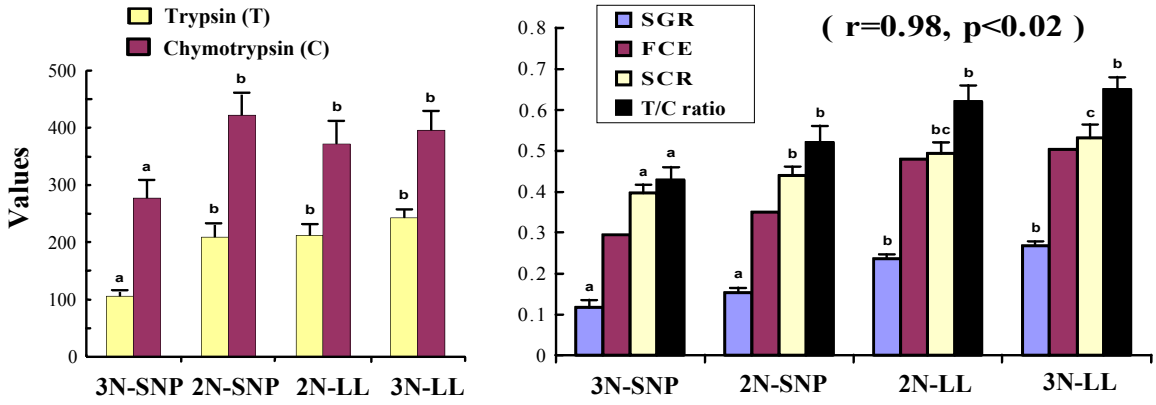

Figure 3. Relationships between specific growth rate (SGR: % day^{-1}), protease specific activities of trypsin and chymotrypsin, feed conversion efficiency (FCE) on tank basis (statistical analysis could not be performed), specific consumption rate (SCR), and the protease activity ratio of trypsin to 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°C in seawater tanks under simulated natural light period (SNP) and 24 h light regime (LL). The enzyme specific activities are expressed as µmol *p*-nitroaniline h^{-1} mg protein^{-1} in the whole pyloric caeca with food content. On group basis, the correlations (r) between the SGR, FCE, SCR, and the T/C ratio are significant (indicated in the bracket), regardless of ploidy and light regime. The same parameter with different superscripts are significantly different ($p<0.05$ by ANOVA). Data were adapted from Sunde *et al.* (submitted revised version).

significant ($r=0.98$, $p<0.02$, Fig. 3). It is interesting to note that the diploids improved growth in the continuous light regime through an increase in T/C ratio value by a reduction in chymotrypsin specific activity (Fig. 3), similar to the observations in Table 1 that higher growth fish had lower chymotrypsin specific activity. The T/C ratio value may be an important parameter for studying specific growth rate through food utilisation especially when genetically manipulated animals are concerned, as enzyme secretions may not be directly comparable between genetically manipulated and ordinary animals. Comparison at a comparable size between fast growth transgenic coho salmon (0+) and control group (1+) showed that a higher T/C ratio value in the transgenic group (1.67), compared to the control (1.23) indicates a potentially higher growth rate of the transgenic fish, while the specific activities of trypsin (4.09±0.18 and 4.40±0.41 U g^{-1} protein, respectively) and chymotrypsin (2.45±0.25 and 3.59±1.11 U g^{-1} protein, $p<0.05$) were not directly comparable (The data were provided by Dr. P. Blier, Département de Biologie, Université du Québec à Rimouski, Canada, personal communication).

Discussion

This presentation is an example showing different possibilities for fish to adapt themselves according to the environmental conditions, either by changing their gene expressions at both molecular and protein levels or by migrating to an environment suitable to their genetic features. Gene expressions of individuals can be different from their ancestors if they have been living in different environments where temperature

and/or food availability are different. This means that genetic structure of any population is dynamic in nature, and it can occur in both aquaculture and natural ecosystem.

A lot of works have been attempted to study the interaction between escaped farmed fish and native conspecifics. Some works showed the possibility of interbreeding between farmed escapees and wild fish (Mork, 1991; Crozier, 1993; Wilson *et al*., 1995; Clifford *et al*., 1998), and there was no geographical isolation to prevent spawning between them (Thorstad *et al*., 1998). Some works suggested the possibility of escaped farmed salmon having lower spawning success compared with wild fish (Lura and Saegrov, 1991; Okland *et al.*, 1995; Fleming *et al*., 1996; Berejikian *et* al., 2001; Youngson *et al*., 2001), but the reproductive success of escaped salmon increases with the time the fish have lived in nature before maturing sexually as cultured females released in nature at the smelt stage had reproductive success similar to that of wild females (Jonsson, 1997). Hard *et al*. (2000) reported morphometric differences between wild and captively reared adult coho salmon and suggested that at least some of the shapes were environmentally induced. Fanning of Atlantic salmon generates rapid genetic change, as a result of both intentional and unintentional selection in culture, which alters important fitness-related traits (Fleming and Einum, 1997). Ruzzante (1994) demonstrated that the direction and intensity of any phenotypic change in food-related agonistic behavior following natural or artificial selection for rapid growth would depend on the relationship between agonistic behavior and access to food, and this balance between competitive (repelling) and antipredator (attracting) tendencies can be altered by domestication, and suggested that such behavioral alterations may affect survival of wild populations if domestic stocks were allowed to interbreed with wild stocks. Johnsson *et al*. (2001) suggested that environmental effects of culture override genetic effects as time in captivity increases, and domestication selection may thus alter reaction norms of farmed animals over environmental gradients and time. The farmed/sea-ranched strain grew faster than the wild one (Einum and Fleming, 1997; Petersson and Jarvi, 2000; Hedenskog *et al*., 2002). Domesticated fish were superior competitors (Rhodes and Quinn, 1998), even in the absence of an initial size advantage, which commonly gives a further advantage to hatchery-raised fish in natural streams (Reinhardt *et al*., 2001), and they could produce long-term genetic changes in natural populations affecting both single-locus and high-heritability quantitative traits, e.g. growth and sea age of maturity (McGinnity *et al*., 1997).

If we look at all these results in a different point of view and based on the present presentation on environmental induced different trypsin expressions at both molecular and protein levels, the escaped farmed salmon and the native conspecifics as well as their oppsprings will have to be able to adapt themselves according to changing in environmental conditions over a period of time for survival. We know that evolutionary changes could accumulate with time in nature, as well as in the culture system as suggested by Fleming *et al*. (1994). 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 or wild fish living in the same environment will have to adapt themselves accordingly for survival and will, with time, become the same population with genetic diversity.

In fact, disease/lice infected farmed escapees and genetically modified fish as well as humans can have negative impact on native fish populations. Disease/lice infected farmed escapees are the obvious problem, and are not included in the present discussion. Cotter *et al*. (2000) suggested the potential for triploids as a mean of eliminating genetic

interactions between cultured and wild population, and of reducing the ecological impact of escaped farmed fish, as triploids had a low return rate to the coast and to freshwater and had inability to produce viable offsprings. This is different from the present point of view. As it is demonstrated in the present presentation that, under a more favourable condition for growth, triploids have higher consumption rate for higher growth rate, unlike diploids that can have better food utilization at a similar consumption rate for higher growth. Triploids may have a higher survival rate in a favourable condition in nature than diploids or wild fish, as they are competitors for food availability and if they spawn, hatching success and survival rate of the offsprings will be low due to low gamete quality for reproduction in triploid fish. This could result in a smaller population of the new generation in that environment in the wild. Although triploids were reported to have a lower survival in seawater under culture conditions, triploids performed better than diploids in terms of growth, while similar freshwater survival between triploids and diploids were observed, except that survival was lower in the triploids for the developmental interval between fertilization and first feeding (O'Flynn *et al*., 1997). Hedrick (2001) also concerned about the ecological and genetic effects of genetically modified organisms, especially on transgenic growth hormone genes. By using a deterministic model, it was shown that if transgenic growth hormone genes have a male-mating advantage and a general viability disadvantage, the increase in the frequency of the transgene would reduce the viability of the natural population and increase the probability of extinction of the natural population.

Recruitment or enhancement of salmon stocks either by releasing farmed salmon or sea ranching is an important stock management programme. It is important that the cultured salmon using for stock enhancement should be produced in the condition similar to the ecological condition where they will be released in order to increased survival rate. Escaped farmed salmon adapt well to the wild life in the ocean, as there was no difference in condition factor, number and weight proportions of prey, or in diet between wild and escaped farmed salmon (Jacobsen and Hansen, 2001). Survival of smelts was higher than the average survival of wild salmon (Dempson *et al*., 1999). Catches were increased due to increased proportion of salmon of farmed origin in the fishery (Hansen *et al*., 1999). Eriksson and Eriksson (1993) reported the success of salmon release programmes over decades with high survival rates of stocked fish and increased natural production of salmon from the Baltic salmon rivers, until an offshore drift gillnet fishery has been developed. Efforts to conserve the remaining wild populations through fisheries regulation appear to be having a positive effect on spawner abundance (McKinnell and Karlstrom, 1999). Wilzbach *et al*. (1998) reported that humans affect the Atlantic salmon ecosystem deleteriously through landscape alteration, exploitation, external inputs, and resource competition. An intact ecosystem provides positive feedback to society by providing food, ecosystem services, and improving the quality of life. Humans become the main problem to nature due to their over-exploitation of the natural resources and their interruption of the natural evolutionary process through alteration of ecological system as well as production of genetically modified fish in aquaculture by utilizing new technology before knowing how to control it.

It may not be easy to avoid the escape of farmed salmon, and if it happens the farmed escapees should not be a treat to the wild populations as long as they are healthy and not genetically manipulated. 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 peacefully.

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