Macrozooplankton as feed source for farmed fish – growth, product quality and safety

Jorma Suontama

Dissertation for the degree of philosophiae doctor (PhD)

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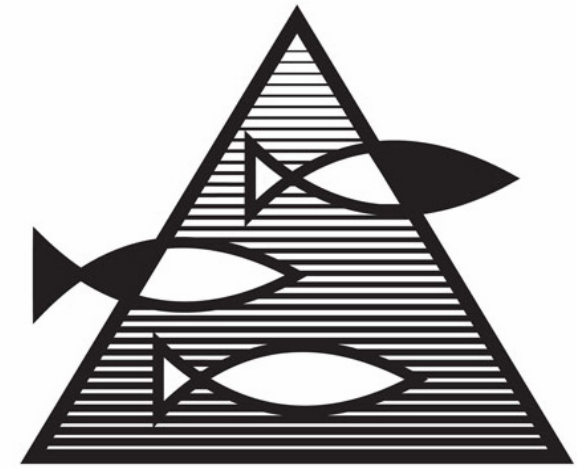
Zooplankton is found in all oceans in the world and in terms of biomass, likely the most abundant and successful animal species on the planet (Wikipedia).



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“*Herra, tee minusta rauhasi välikappale, niin että sinne missä on vihaa, kylväisin rakkautta, missä katkeruutta, toisin anteeksiantamusta, missä epäsopua, loisin yhteisymmärrystä, missä erehdystä, viittaisin totuuteen, missä epäilystä, auttaisin uskoon, missä epätoivoa, kantaisin toivoa, missä pimeyttä, loisin sinun valoasi, missä alakuloisuutta, virittäisin ilon. Niin että, oi mestari, en yrittäisi niin paljon etsiä lohdutusta kuin lohduttaa toisia, pyytää ymmärtämystä kuin ymmärtää toisia vaatia rakkautta kuin rakastaa toisia. Sillä antaessaan saa, kadottaessaan löytää, antaessaan anteeksi saa itse anteeksi, kuollessaan nousee iankaikkiseen elämään."* -Saint Francis of Assisi (1182-1226).

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List of papers

I. Olsen, R.E., **Suontama, J**., Langmyhr, E., Mundheim, H., Ringø, E., Melle, W., Malde, M.K. & Hemre, G.-I. (2006) The replacement of fishmeal with Antarctic krill, *Euphausia superba* in diets for Atlantic salmon, *Salmo salar*. *Aquaculture Nutrition*, **12,** 280-290.

II. **Suontama, J**., Kiessling, A., Melle, W., Waagbø R., Mundheim, H., Olsen, R.E. (2006) Protein from northern krill (*Thysanoessa inermis*), Antarctic krill (*Euphausia superba*) and the Arctic amphipod (*Themisto libellula*) can partially replace fish meal in diets to Atlantic salmon (*Salmo salar*) without affecting product quality. *Aquaculture Nutrition*, (in press).

III. **Suontama, J**., Karlsen, Ø., Moren, M., Hemre, G.-I., Melle, W., Langmyhr, E., Mundheim, H., Ringø, E., Olsen, R.E. (2006) Growth, feed conversion and chemical composition of Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.) fed diets supplemented with krill or amphipods. *Aquaculture Nutrition*, (in press).

IV. Ørjan Karlsen, **Jorma Suontama** & Rolf Erik Olsen. (2006) Effect of Antarctic krill meal on quality of farmed Atlantic cod (*Gadus morhua* L.). *Aquaculture Research*, (in press).

V. Moren M., **Suontama J.**, Hemre, G.I., Karlsen, Ø., Olsen, R.E. & Julshamn, K. (2006) Element concentrations in meals from krill and amphipods, — Possible alternative protein sources in complete diets for farmed fish. *Aquaculture*, (in press).

In the thesis the papers are referred to in the text by their Roman numerals.

1. Introduction

Aquaculture is currently the most rapidly growing animal food-producing sector on a global basis. Since 1970, the average production rate has increased by more than 8% per year, compared to 1.2% for capture fisheries and 2.8% for terrestrial farmed meat-production systems (FAO, 2004a). Norwegian aquaculture production is based mainly on Atlantic salmon where production relies heavily on being supplied with fish meal and oil as the major feed ingredients. In 2004, Norway consumed 309 000 tonnes of fish meal and was ranked the fifth largest fish meal consumer in the world (IFFO, 2005). Atlantic salmon production alone required 780 000 tonnes of feed in Norway in 2005 (Kjønhaug, 2006). It is estimated that salmon production alone required 19.5% and 51% of reported global fish meal and –oil production, respectively in 2003 (FAO, 2005a). In addition, the production of Atlantic cod is increasing in Norway. It has been ranked to have the best growth potential of all aquaculture species (FAO, 2004a), and compete for the same marine protein and oil sources as salmon in their feeds.

As many seas are already over exploited (FAO, 2004a), there is an increased concern that the growth in production cannot be supported with increases in input of the traditional marine resources (Waagbø *et al.,* 2001; New & Wijkström, 2002; Tacon, 2004). Lack of suitable raw materials for fish feed may be even more critical in the years to come, as more fish oil is being used in human nutrition. It is also possible that similar trends will be seen with fish meal (Naylor *el al.* 2000).

The cost of fish meal has increased steadily over the years. Consequently, the amount of fish meal in feeds has decreased from 54% to ca. 30% over the past 5-6 years (Bjordal, 2006) with subsequent replacements with cheaper feed raw materials, mainly from plant origin. However, carnivorous fish species are not well adapted to eat vegetable sources as the natural diet for carnivorous fish consists mostly of fish and crustaceans. Additionally, vegetable meals do not necessarily meet the nutritional demand of fish having unbalanced amino acid composition, often high level of

indigestible carbohydrates and various antinutrients, while the oil sources lack the long chain ω-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), essential for growth for marine fish species (Sargent *et al.,* 2002). High inclusion levels of ingredients of vegetable origin in feed for carnivorous fish species could result in morphological changes in the intestine (Baeverfjord & Krogdahl, 1996), lower muscle ω-3 fatty acid level and thus lower health benefit for humans (Bakke-McKellep *et al.,* 2000).

When seeking alternatives to fish meal and oil, one solution may be harvesting zooplankton from lower trophic levels of the marine ecosystems. These resources are presently unexploited yet abundant, and estimated annual production is much higher than for future fisheries. Zooplankton has great harvesting potential (Nicol & Endo, 1997) and is not presently used for human food. Nutritional values of many of these organisms are reported to be similar to fish meal (Storebakken, 1988).

In North Atlantic waters zooplankton resources include various krill species, Arctic and boreal species of amphipod and copepods like *Calanus finmarchichus*. In the Southern Ocean, zooplankton consists mainly of Antarctic krill. Lately, interest in developing these species for fishery has increased. The Antarctic krill's biomass is estimated to be between 125 to 725 million tonnes (FAO, 2005b). Harvesting only a few percent of this enormous production would easily give enough raw materials to supply the aquaculture industry with raw materials for many years to come.

The aim of this thesis was therefore to study marine resources from lower trophic levels in terms of macrozooplankton as fish meal replacers in feed for three farmed coldwater fish species. In order to replace fish meal with macrozooplankton meal, it is essential to know the consequences of both type and inclusion level of the replacers on fish growth, fish health, chemical and sensory fillet quality and consumers' safety.

2. The aims of the thesis

In the feeding experiments in this thesis, meals prepared from krill and amphipods were used as sources of protein in diets for Atlantic salmon, halibut and cod. The aims were to study the effects of these meals as fish meal replacers on:

- Diet properties (pellet hardness and lipid coating) (**Paper I** and **III**).
- Feed conversion and growth rate (**Paper I, III** and **V**).
- Macro nutrient digestibility (**Paper I** and **III**).
- Muscle chemical and technical quality (**Paper I, II** and **III**).
- Fillet sensory quality (**Paper II** and **IV**).
- Undesired element concentrations in meals, diets, and farmed fish fillets after feeding (**Paper V**).
- Health status of fish (**Paper I, III** and **V**).

In this thesis, I aimed to study the optimal inclusion level of different macrozooplankton meals in extruded feeds, and to produce diets that more closely resemble the natural diets of the common Norwegian aquacultured species. Only a few studies have investigated diets for salmon cod and halibut using alternative marine ingredients. It is important to verify if macrozooplankton constitute sustainable and long-term alternatives to high quality fish meal without compromising fish production, as well as fillet quality or safety.

3. Background

3.1. Macrozooplankton

Crustaceans are large group marine invertebrate (arthropods) constituting of approximately 55 000 species including among others familiar animals such as lobsters, crabs, shrimp and zooplankton. Commonly, crustaceans have a stiff exoskeleton protecting the the animal. Zooplankton is a group of crustaceans that have limited ability to position in the ocean currents. They feed on other plankton and form a link between phytoplankton and higher organisms in the pelagic food web. Planktonic animals are classified depending on their size, and krill and amphipods are two groups of macrozooplankton. They represent natural feed organisms for many fish species during parts of their life cycle (Grønvik & Klementsen, 1987; Dalpadado & Bogstad, 2004). Total standing biomass of krill (Euphausiacea) in the Nordic and Barents Seas has been estimated to be between 91-161 M tonnes with an annual production of 242 M tonnes (Table 1), and in the Southern Ocean between 125 to 725 M tonnes (FAO, 2005b) – up to seven times the total present marine harvest of capture fisheries (FAO, 2003). In addition there is a substantial annual production of amphipods and copepods (Table 1). Zooplankton is located very dispersed on large areas of marine environment making the abundance estimations difficult. There are some variations in reported abundance estimations between studies as seen in Table 1.

Table 1. *Biomass and production (wet weight) of macrozooplankton standardised to an area of 3.1 million km^2, corresponding to the Norwegian and Barents Seas and eastern parts of the Greenland and Iceland Seas (Melle & Olsen, pers. comm.).*

Species/group	Biomass (mill tonnes)	Production (mill tonnes)	Original area (mill km^2)	Source
Euphausiids (krill)	91		1.7	Dalpadado *et al.,* 1998
Euphausiids (krill)	161	242*	3.1	W. Melle, unpublished results
Amphipods	201		1.7	Dalpadado *et al.,* 1998
Amphipods	49	74*	3.1	W. Melle, unpublished results
Calanus finmarchicus (copepod)	22**	88	2.9	Aksnes & Blindheim, 1996
Calanus ssp.	30-125	120-500**	3.1	Hassel & Melle, 1999
Calanus ssp.	75	298**	3.1	Holst *et al.,* 2000

* Based on P/B=1.5 (Sakshaug *et al.,* 1994)

** Based on P/B=4 (Sakshaug *et al.,* 1994)

*** The area represents main distributional area for the species.

Below is a short introduction to the biology and abundance of krill and amphipod species that were used in the feeding studies described in this thesis.

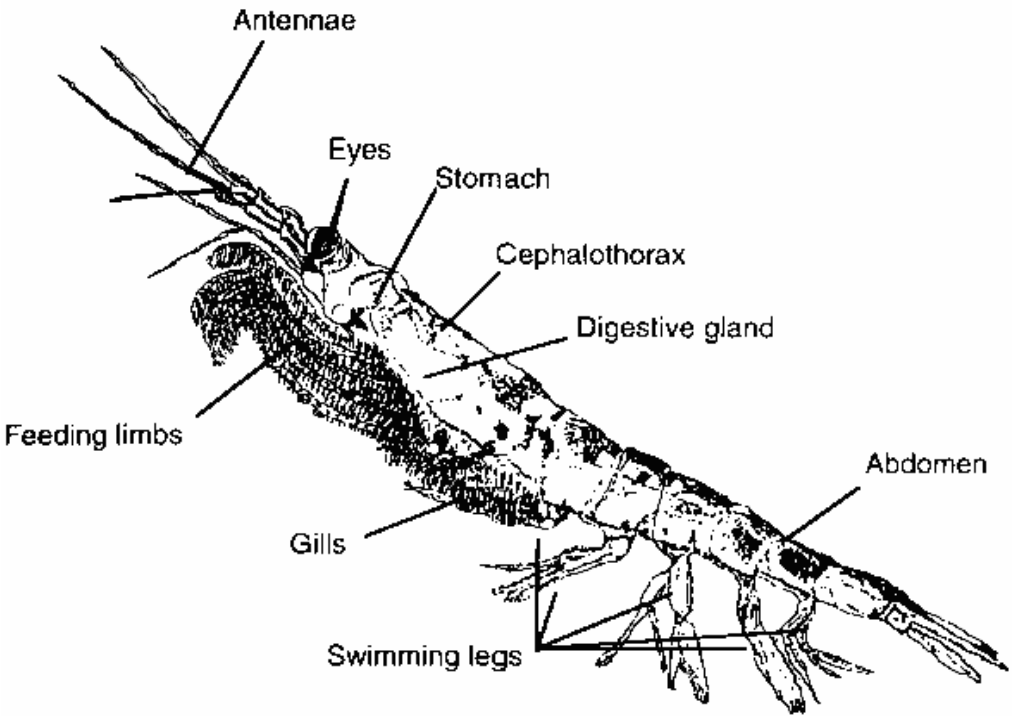
3.1.1. Antarctic krill, *Euphausia superba*

Antarctic krill *E. superba* is a shrimp-like crustacean playing a significant role in the pelagic ecosystem as food for fish, sea birds and sea mammals in the Southern Ocean round Antarctica. The morphology of a typical krill is shown in Figure 1. All Euphausiaceas have gills clearly visible below the cephalothorax and eyes at the front of the head; the stomach is behind the eyes and they have a characteristic big muscle system from the middle to the back of the body (Ellingsen & Mohr 1980a; Everson,

2000). *E. superba* is the best characterized of all 85 known Euphausiid species. They tend to form dense schools in continuous layers, often staying deep during daytime and ascending at night. Their length varies from 1.5 to 6.0 cm, and mature animals have a live weight of around 1.0-1.5 g. Antarctic krill has a high nutritional value (Grantham, 1977; Budzinski *et al.,* 1985) and a wide range of products has been developed from it (Eddie, 1977; Everson, 1977; Grantham, 1977; Suzuki, 1981; Suzuki & Shibata, 1990).

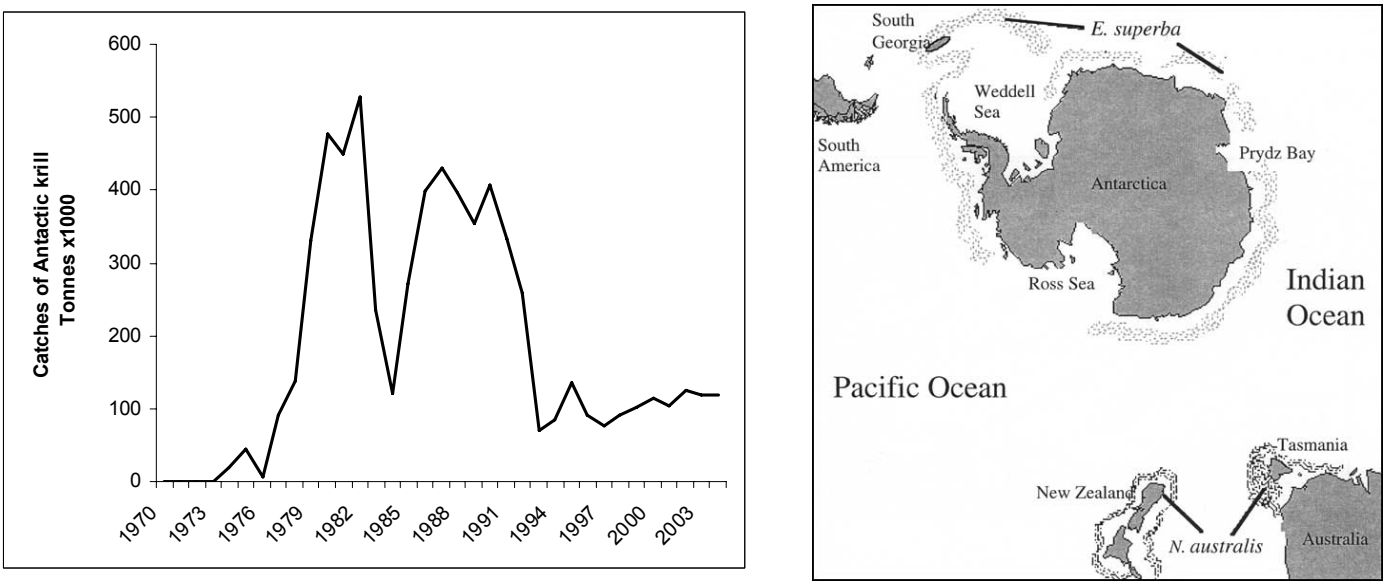
Experimental krill fishing began in 1961-62 with a catch of 4 tonnes by the former USSR. Commercial catching started during the season of 1972-73. The total catch reached a maximum of 477 184 tonnes in 1980, and 528 201 tonnes in 1981-1982, before declining to a stable level of around 100 000 tonnes since then (128 218 tonnes in 1984, and 83 962 tonnes in 1994) (Figure 2 A). Krill catches dropped due to lack of economic profitability, the collapse of the former USSR and processing problems possibly associated with the discovery of high levels of fluoride in the exoskeleton of krill (Nicol & Endo, 1997; Ichii, 2000).

The Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) Scientific Committee has suggested a precautionary catch limit of 4 M tonnes of krill for the Atlantic Sector (Area 48) (Hewitt *et al.,* 2002). Harvesting of 4 M tonnes would supply nearly 400 000 tonnes of marine protein and 80 000 tonnes of marine lipids. In 2003/2004 less than 120 000 tonnes were exploited (CCAMLR 2005). For the season 2004/05 about 160 000 tonnes were estimated to have been caught (SC-CCAMLR 2005a). Japan, South Korea, Poland and Ukraine have been the main harvesting countries and it is likely that they will continue this fishery (Nicol & Endo, 1997). However, it is probable that international companies will also take part in harvesting as catching efficiency has improved (Nicol & Foster, 2003). The krill fishing area round Antarctic corresponds to about four times the area of Australia. Distribution of Antarctic krill is shown in Figure 2 B.



Source: FAO, 1997

Figure 1. *Typical morphology of krill.*



A **B**

Figure 2. *Reported annual catches and distribution of Antarctic krill (E. superba) in Southern Ocean (FAO, 1997; 2005b).*

3.1.2. Northern krill, *Thysanoessa inermis*

T. inermis is a common krill species over the shelves surrounding the deep basins of the Nordic Seas (Norwegian, Iceland and Greenland Seas) and Barents Sea (Dalpadado *et al.,* 1998; Dalpadado, 2006) (Figure 3). Adult organisms of *T. inermis* reach the size of 1.5-3.5 cm. *T. inermis* is prey to a wide range of plankton-feeding fish including herring, mackerel and blue whiting (Melle *et al.,* 2004) and marine mammals (whales and seals). There are indications that fishing stocks needs to have a certain krill swarming density and biomass to perform good growth and to reach high condition index. *I.e.* it is reported, that there is a reduction in the condition factor of herring at years when the production of copepods are low (R. Toresen, IMR, Bergen, pers. comm.). That means that the ecosystem is vulnerable for biomass fluctuations, where high outtake of one species will directly affect the biomass of the others. The standing stock of krill in the Norwegian Sea has been estimated to be 42 M tonnes and annual production about twice this amount (Melle *et al.,* 2004). Furthermore, it is uncertain if zooplankton in the Norwegian or Barents Sea forms economically profitable swarms.

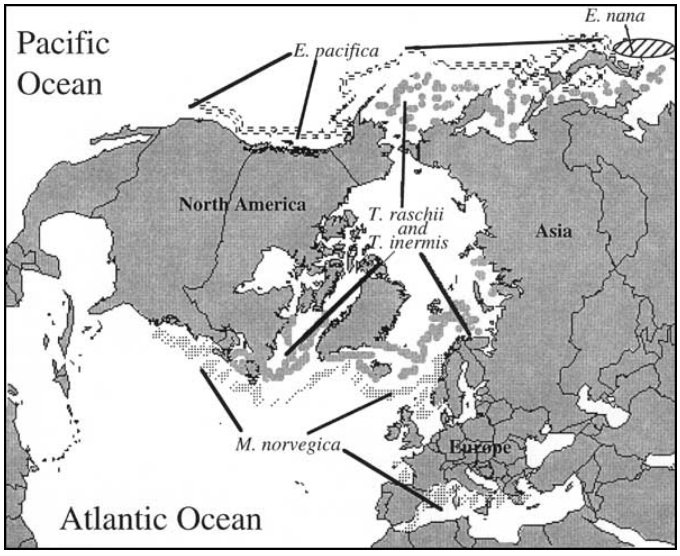


Figure 3. *Distribution of northern krill (FAO, 1997).*

3.1.3. Arctic amphipods, *Themisto* ssp.

The amphipods are considered to be the third largest group of zooplankton in Arctic waters. In the Barents Sea there are some quantitatively important species in the genus *Themisto*. *T. libellula* is classified as hyperiid, being carnivore, preying mostly on *Calanus* ssp. (Dalpadado *et al.,* 2002). The standing stock of amphipods of the Norwegian Sea has been estimated to be 29 M tonnes, and the annual production about twice this level (Skjoldal *et al.,* 2004). They reach a length of 2-4 cm and are important food items for many important fish stocks such as blue whiting, capelin, herring, salmon and cod (Dalpadado & Bogstad, 2004). *T. libellula* may play a somewhat similar role to that of Antarctic krill (*E. superba*) in the Southern Ocean, being preyed upon by marine mammals, birds, fishes and squid (Melle *et al.,* 2004).

3.2. Chemical composition of zooplankton

Macrozooplankton is rich in crude proteins 60-78% of dry matter (dm), crude fat 7-30% (dm) and 12-17% ash (dm), and a number of reports have been made on the chemical composition, mainly of Antarctic krill (Martin, 1979; Siebert *et al.,* 1980; Suzuki, 1981; Ellingsen, 1982; Storebakken, 1988; Everson, 2000; Ju & Harvey, 2004). The chemical composition of some macrozooplankton is presented in Table 2. Macrozooplankton also contains natural carotenoids, mainly astaxanthin (Table 2; reviewed by Storebakken, 1988). Zooplankton and other crustaceans were used as a source of pigment in early days of salmonid farming, but were later replaced with synthetic pigment sources for more predictable muscle pigmentation (Tsukuda & Amano, 1965; Spinelli, 1974; 1979; Torrissen, 1989). Additionally, increasing products have been developed from zooplankton other than aquaculture purposes including nutriceutical, cosmetic and pharmaceutical fields (Hamovitch, 2001).

Table 2. *Wet weight biochemical composition of raw zooplankton caught in May 2003 (E. Langmyhr 22 Feb 2006, Norwegian Institute of Fisheries and Aquaculture Research, Bergen, Norway).*

	T. inermis	*T. libellula*	*E. superba*
%			
Crude protein	16	12	13
Moisture	76	79	82
Ash	3	5	3
NaCl*	1	1	1
Fat	5	3	2
Chitin	0.5	2.3	0.6
mg $100g^{-1}$			
TMAO-N**	104	105	110
mg kg^{-1}			
Astaxanthin			
Free	3	1	5
Ester	15	1	32
Total Astaxanthin	18	2	37

* = mainly from sea water

** = trimethyl-amine N-oxide

Crude protein that originates from crustaceans often contains non-soluble nitrogen that originates from the exoskeleton of these organisms. In Antarctic krill this may contribute for up to 20% of the total protein (Pierce *et al.,* 1969; for review, Storebakken, 1988). The carapace fraction also contains an adequate content of amino acids (approxmately 66% of crude protein; Siebert *et al.,* 1980). In general, it appears that the overall composition is similar to that of known related species such as shrimps, crab, lobsters, crayfish etc. (Gilberg, 1971; Grantham, 1977). The amino acid composition of krill meal as sole protein source has been reported to satisfy the requirements of essential amino acids of common cultured fish like salmonids, carp

and tilapia (Hertrampf & Piedad-Pascual, 2000; Nicol *et al.,* 2000; Table 3). Fresh caught krill contains a high proportion of free amino acids (6.6-9.5% of dm) (Ellingsen, 1982). The most common free amino acids (85-91%) are taurine, glycine, proline, β-alanine and alanine (Ellingsen, 1982).

Table 3. *Recommended protein and amino acid contents for cultured fish compared to the protein and amino acid levels of Antarctic krill meal, Antarctic krill hydrolysate and anchovy meals. Values are reported as percentage of protein. (Modified from Nicol et al., 2000).*

	Rainbow Trout[1]	Pacific salmon[1]	Common carp[1]	Tilapia[1]	Krill Meal[2]	Krill Hydrolysate [3]	Anchovy meal[1]
Total protein (% dw)	38	38	35	32	55	60	65.4
Agrinine	3.9	5.4	3.7	3.7	5.8	7.0	5.9
Histidine	1.8	1.6	1.5	1.5	1.9	2.8	2.5
Isoleucine	2.4	2.0	2.7	2.7	7.5	5.0	4.8
Leucine	3.7	3.5	3.0	3.0	4.9	8.3	7.7
Lysine	4.7	4.5	4.5	4.5	6.5	6.9	7.7
Methionine + cysteine	2.6	3.6	2.8	2.8	3.8	4.3	4.0
Phenylalanine + tyrosine	4.7	4.6	4.8	4.8	8.9	7.7	7.7
Threonine	2.1	2.0	3.3	3.3	4.0	5.0	4.3
Tryptophan	0.5	0.4	0.9	0.9	ND[4]	1.5	1.1
Valine	3.2	2.9	2.4	2.4	5.3	5.5	5.3

[1] NRC, 1983

[2] Rehbein, 1981

[3] Biozyme Systems, Inc. Internal data. Values are % of total amino acids of *E. superba*

[4] Not determined

The total lipid fatty acid composition in Antarctic krill (*E. superba*) is given by Hertrampf & Piedad-Pascual (2000) and contains 20-42% saturated fatty acids, 35-

50% monounsaturated fatty acid and 18-39% polyunsaturated fatty acid (PUFA). The PUFA consist mainly of EPA (20:5 n-3) and DHA (22:6 n-3) making zooplankton good sources for these fatty acids. The total lipids in fresh caught Antarctic krill consist mostly of triacylglyserols and phospholipids (Ju & Harvey, 2004), while other zooplankton species store lipids also as wax-esters, like *Thysanoessa* (25% of the total lipids) and the amphipod *Libellula* sp. (45% of the total lipids) (Falk-Petersen *et al.,* 2000; E. Langmyhr, Norwegian Institute of Fisheries and Aquaculture Research, pers. comm.). There is however, considerable variation in chemical composition of zooplankton between studies that most likely is due to the complex interaction of season, grazing period, and area where the krill is caught, as well as the age and sex of the animals (reviewed by Grantham, 1977; Shibata, 1983).

The storage conditions affect the chemical composition of zooplankton. Caught raw zooplankton is known to have high autolysis compared to fish (Ellingsen, 1982). Autolysis is even enhanced, if zooplankton has been crushed after the catch (Ellingsen & Mohr, 1987). Prolonged storage time increases the leakage of both water-soluble proteins (Ellingsen & Mohr, 1980a) and lipids presumably from the action of active digestive enzymes (protease, lipases and phospholipases) (Ellingsen & Mohr, 1980b). Thus, zooplankton has to be processed within four hours after being caught at a temperature of 2-4 ºC (Hertrampf & Piedad-Pascual, 2000) or within 6 days when stored at 0 ºC (E. Langmyhr, pers. comm.) to avoid deterioration in quality.

3.3. Undesirable compounds in zooplankton

Marine raw materials, including zooplankton are naturally rich in many elements such as zinc, copper and cadmium (Soevik & Brækkan, 1979, Boone & Manthey, 1983; Ritterhoff & Zauke, 1987; 1988; Petri & Zauke, 1993; Nygård *et al.,* 2001; Edmonds, 2003). In some cases, their content may exceed the EU's current upper limits (European Commision (EC) Directive 2003) for fish feed and feed ingredients,

and restrict their use (see Results and discussion). However, metal concentration varies greatly depending on the species in question, area of origin, grazing period, sex and age of the animal. The relatively high content of fluoride in the exoskeleton, mainly in the form of $Ca_5(PO_4)_3F$ has received particularly much attention. This fluoride compound strengthens the carapace of these animals and constitutes up to 1500 mg kg^{-1} (whole body dry weight) in the Antarctic krill (*E. superba*) and northern krill (*Meganyctiphanes norvegica*) (Hodge, 1965; Soevik & Brækkan, 1979). The fluoride in krill has been shown to have high availibility in humans (Trautner & Siebert, 1986) and high fluoride content in krill was one reason for the collapse of its fishery in the mid '80s. This problem was partly solved later when a technique for peeling crustaceans was introduced. Additionally, Julshamn *et al.* (2004) showed that fluoride is not accumulated in fish when the fluoride content in feed ranged from 18 to 358 mg kg^{-1} and hence does not contribute to human exposure. The EC has set upper limit for fluoride in feed raw materials at 2000 mg kg^{-1} and in animal feed, including feed for farmed fish at 150 mg kg^{-1} (EC Directive 2003). This level is easily exceeded if macrozooplankton meal is used as feed raw materials. Increasing the maximum limit for fluoride in the form of krill meal in complete feedingstuffs is evaluated by the Norwegian Scientific Committee for Food Safety (VKM) not to pose any risk for the consumers (www.vkm.no).

Organic contaminants such as polychlorinated biphenyls (PCBs) and dioxins are resistant to environmental degradation, and have shown to accumulate in the fatty tissues of organisms and biomagnify across trophic levels (Borgå & Guardo, 2005). Hence, accumulations of organic contaminants have shown to be lower in plankton and zooplankton and increasing towards higher trophic levels, being high in fatty fish species and in top predators like sea birds and marine mammals (Borgå *et al.,* 2001). Fish oil represents the source with highest concentrations of these compounds in fish feeds. These compounds are harmful since they accumulate in the human body tissue and may injure the nerve and the immune system and impair reproduction. Newborns of mothers who reported consumption of PCB-contaminated fish from Lake Michigan showed lower birth weights (Fein *et al.,* 1984) and lower IQ scores at

school-age (Jacobson & Jacobson, 1996). Publicity related to organic contaminants in fish muscle has generated much concern lately (Jacobs *et al.,* 2002; Hites *et al.,* 2004). However, the European Food Safety Authority (EFSA, 2005) has recently evaluated the balanced risks and benefits of seafood consumption and concluded that the benefits overcomes the possible negative effects, and one to two fish servings (with high ω-3 fatty acid level) a week have beneficial effects on cardiovascular system and against coronary heart disease.

A major part of the crustaceans' carapace (exoskeleton) is the polysaccharide chitin (poly-*N*-acetylglucosamine). Chitin is analogous to cellulose, except that the hydroxyl group (-OH) on the C-2 position of the glucose molecules in cellulose is replaced by an *N*-acetylamino group on the chitin molecule (White *et al.,* 1968; Jobling, 2001). High dietary chitin and chitosan levels lower the energy available for absorption, elevate the feed conversion ratio (FCR) and reduces growth in aquatic and marine species. This was seen in studies with shrimp (*P. monodon*) and tilapia (*Oreohromis niloticus* x *O. aureus*) where 10% dietary chitin and chitosan levels reduced growth, and depressed protein and lipid digestibility (Shiau & Yu, 1998; 1999). Studies with rainbow trout (*Oncorhynchus mykiss*) have also indicated that dietary chitin at 10 and 30% of the diet was not digested (Lindsay *et al.,* 1987). The study with red sea bream (*Pagellus bogaraveo*), Japanese eel (*Anquilla japonica*) and yellowtail (*Seriola lalandei*), on the other hand, did not show any growth suppression at 10% dietary level of chitin, chitosan or cellulose (Kono *et al.,* 1987). Various fish species clearly perform differently to dietary amount and source of chitin.

3.4. Zooplankton as feed ingredient in aquaculture

The potential use of zooplankton as a feed ingredient was the focus of several studies in the late '70s and early '80s (for a review, see Storebakken, 1988). These studies were mainly performed using Antarctic krill as feed source and rainbow trout as the experimental fish. Most results showed that partial fish meal replacement with krill

either increased the growth (Pfeffer & Becker, 1977; Jahn *et al.,* 1978; Koops *et al.,* 1979) or had no negative effect on growth (Beck *et al.,* 1977). When full replacement was tested, some studies reported of reduced growth rates, (Beck *et al.,* 1977, Koops *et al.,* 1979), whereas others found no negative effects (Vens-Cappell & Horstmann, 1978; Papuktchieva *et al.,* 1981). These studies were however performed at a time when aquaculture was still young, and farming conditions and feed technology were still developing. At that time the farmed fish displayed significantly lower growth rates and feed that was used was mostly moist feed. In this respect these data may be hard to compare to current aquaculture settings, and with other species.

Post-larval chum salmon (*O. kisutch*) (0.41 g) reared in sea-water showed significantly higher weight gain, feed conversion efficiency and survival when Antarctic krill meal was used as fish meal replacement for up to 75% of the diet (Roem & Kelly, 1991). Similarly, Anderson *et al.* (1997) reported higher weight gain in juvenile chinook salmon (*O. tshawytscha*) (initial weight of 2.83 g) in a 56-day study when air-dried krill meal replaced fish meal at 0 to 25% dm (0-36% of the protein) in the diet. Recently, Julshamn *et al.* (2004) reported no effect on growth rate or feed conversion (FCR) with the diets where up to 30% of fish meal was replaced with Antarctic krill meal in Atlantic salmon (0.5 kg) diets. Also, no effect on weight gain, feed intake or specific growth rate (SGR) was seen in juvenile rainbow trout (initial weight of 5.5 g) when fish meal was replaced with Antarctic krill meal up to 15% of the diet (Yoshitomi *et al.,* 2006). Ringø *et al.* (2006) reported that substituting 50% of the fish meal protein with northern krill (*M. norvegica*) did not have any negative effect on growth performance or feed conversion in 100g Atlantic salmon during a 46 days feeding experiment.

Krill products have been reported to function as excellent feed attractants in diets for species like red sea bream, Japanese eel and gray mullet (*Mugil cephalus*) (Allahpichay *et al.,* 1984a; 1984b) and juvenile rainbow trout (Oikawa & March, 1997). The inclusion of freeze-dried krill and krill meal (up to 75% of the diet) in the diet made it more acceptable and proved to be the most attractive starter diet for

largemouth bass (*Micropterus salmoides*) (Kubitza & Lovshin, 1997) compared to fish fed control diet based on fish meal. Improved diet ingestion rates were also obtained in yellow perch (*Perca flavescens*) and lake whitefish larvae (*Coregonus clupeaformis*) by including krill meal as attractants in the diet (Kolkovski *et al.,* 2000).

Krill has also been investigated in sensory quality studies (flavour, texture, fillet colour) using rainbow trout as the experimental fish. These studies showed that inclusion of krill in feed either enhanced (texture and flavour) (Spinelli, 1979) or did not have any effect on the organoleptic quality (Reinacher, 1979). Krill was formerly used as a pigment source in salmonids and red sea bream farming (Tsukuda & Amano, 1965), but was replaced later with synthetic astaxanthin sources. Astaxanthin in crustaceans is bound in the indigestible exoskeleton. Satisfactory salmonid muscle pigmentation would require high dietary levels of crustacean meals that increase simultaneously the diet indigestible matter. Astaxanthin level and availability also varies between different crustaceans species. With synthetic astaxanthin more predictable and effective muscle pigmentation is achieved than using astaxanthin in form of crustacean meals.

3.5. Dietary protein quality and growth

Carnivorous fish like Atlantic salmon, halibut and cod have a metabolism adapted to high protein, variable lipid intakes (Pike *et al.,* 1990) and low carbohydrate intakes (Stone, 2003). High protein requirement in carnivorous fish species can be explained by their low ability to use endogenous amino acids in protein re-synthesis; it has been suggested that only 50% of the endogenous amino acids are retained in the fish muscle compared to 70-80% in humans (Espe *et al.,* 2001). Besides, fish have an effective mechanism to excrete surplus nitrogen over the gills in the form of soluble ammonium compounds (Pike *et al.,* 1990).

The growth process requires that the protein synthesis exceed the catabolism in the whole body (Houlihan *et al.,* 1986). Improved efficiency of converting feed into growth seems to relate to lower protein degradation grade rather higher protein synthesis (Carter *et al.,* 1993). Variation in protein retention seems to be as high between individuals as it is between the different body tissues. In salmonids, retention efficiency estimates from synthetised proteins range from 23-62% (Houlihan *et al.,* 1995; Owen *et al.,* 1999). In what degree the synthetised protein is retained within a muscle depends on the amino acid composition of the protein source, protein type and length, digestible energy intake, presence of possible anti-nutritional factors and the amino acid requirement of fish species in question. Protein quality in different meals may vary considerably depending on the source of raw materials, freshness, manufacturing process, and whether the meal is made from whole animal or from waste and by-products (Tarr & Biely, 1972).

Potential fish meal replacers may be limiting in some essential amino acids. Methionine is assumed to be the first limiting amino acid in fish (Savage & Foulds; 1987; Lied & Berge, 1995), and lysine is easily destroyed at high manufacturing temperatures. Limiting amino acids may be supplemented with crystalline amino acids (Mambrini *et al.,* 1999), at least up to 10% of dietary protein (Espe *et al.,* 2006) or by combining raw materials with complementing amino acid profile (Yamamoto *et al.,* 1995). Fish cannot store diet amino acids, and diet imbalanced amino acid composition will result in higher catabolism and reduced protein utilisation and growth. The knowledge of the total amino acid requirement of fish is still not well understood. Estimated amino acid requirement is often based on the amino acid composition of the whole fish or fillet (NRC, 1993). Better understanding of nutritional requirement of fish will improve sustainable use of feed resources.

Alternative ingredients for fish meal in fish feeds can be evaluated by digestibility estimations either *in vitro* using individual proteolytic enzymes or combination of them in a closed system (Pedersen & Eggum, 1983; Dahlin & Lorenz, 1993; Anderson *et al.,* 1993) or *in vivo* methods measuring digestibility in mink

(*Mustela vision*) (Romero *et al.,* 1994) or in fish (Anderson *et al.,* 1992; Bureau *et al.,* 1999). Furthermore, protein quality can be assessed by growth studies with comparative slaughter (Pfeffer *et al.,* 1994; Bureau *et al.,* 2000) or combination of digestibility and growth methods (Gomes *et al.,* 1995; Mambrini *et al.,* 1999).

In vivo protein quality studies commonly compare the relation of the dietary nitrogen/protein content to the retained nitrogen/protein in the body. The protein content in animal feed is traditionally calculated from the total nitrogen (N) amount (N × 6.25) based on the assumption that protein contains 16 percent nitrogen (NRC, 1993).

Common methods to study protein quality (Espe *et al.,* 2001; Jobling, 2001):

- Apparent digestibility (AD): dietary N concentration is compared to the concentration of N in the faeces, and related to the concentration of an inert indigestible marker in feed and faeces.

- Net protein utilisation (NPU): the amount of excreted N in faeces is compared to the retained N in the muscle.

- Protein efficiency ratio (PER): the animal live weight gain is compared to the amount of ingested protein.

- Protein productive value (PPV): the amount of retained protein in the body is compared to the amount of ingested protein.

Crustacean meals have high crude protein content, but high level of proteins may origin from the exoskeleton constituting high non-soluble nitrogen fraction in these meals. High non-soluble matter in the diet may affect elevated faecal water content and reduced fat digestibility, as observed in salmonids when fed with high dietary non-soluble nitrogen originating from soy meal (Refstie 1997; 1999) and in tilapia fed with 10% chitin and chitosan in the diet (Siau & Yu, 1999). Spinelli *et al.* (1979) reported lower muscle proximate lipid content in rainbow trout when moist pellet included 7.5% *E. pacifica* meal instead of tuna viscera. Lower compositional lipid

level might be a result of faster movement of digesta through the gastrointestinal tract and hence lower lipid absorption. However, the level of chitin in final feeds was not mentioned in that study.

Anderson *et al.* (1993) showed that there was a significant difference in protein quality in various of fish meals depending on the meal source and manufacturing conditions. Even higher variability in protein digestibility is seen between animal and plant ingredients measured in different fish species (for review, Jobling *et al.,* 2001). Norwegian low temperature (LT)-fish meal and cod muscle have been shown to have high digestibility in Atlantic salmon (87% and 93%, respectively) (Anderson *et al.,* 1995; Espe *et al.,* 1993). The amino acid compositions of northern krill meal (*M. norvegica*) and Antarctic krill meal (*E. supreba*) are shown in Table 3 and these have been shown to satisfy the essential amino acid requirement of salmonids, carp and tilapia (NRC, 1993; Storebakken, 1988). However, studies considering digestibility and utilisation of proteins from zooplankton are limited. Vens-Cappell & Horstmann (1978) reported that the digestibility of the crude protein from whole krill meal in trout was 87% and for amino acid proteins it was 92%.

Feed intake explains 70-80% of measured growth variation in fish (Rosenlund *et al.,* 2005). Therefore, the palatability of feed is of primary importance. Raw material free amino acid content or pre-hydrolysis of raw materials may increase the feed intake and improve the growth (Espe *et al.,* 1993).

Generally, fish in their early life stages have a higher protein requirement than adults (Wilson, 2002). Small fish use dietary protein primarily for lean muscle growth, whereas, bigger sized fish deposit relative higher levels of dietary lipids in their muscle (Brett, 1979). Cod on the other hand store surplus energy in the liver. Small fish use relative lower level of energy (lipids) for swimming and maintenance, whereas bigger fish use more energy for basal metabolism and swimming, where higher dietary lipid levels can be used as energy source. Hence, diets are developed to meet the demand for fish in different life-stages generally changing the protein/lipid ratio. Current commercial salmon grower diets contain 34-47% protein and 28-40%

lipid (Refstie, 2001), and the optimal diet for cod should include 50-60% protein, 13-20% lipid and less than 15% starch (Hemre *et al.,* 2004).

3.6. Need for fish meal replacements

High quality fish meal is considered as the ultimate protein source for carnivorous fish species because of its well-balanced amino acid content, good digestibility and palatability, low crude fibre content and absence of anti-nutritional factors (Rumsey *et al.,* 1993). That makes intensive carnivorous fish farming dependent on the use of fish meal as the sole or major source of dietary protein in formulated diets (Tacon, 1994). Traditional fish meal is prepared from dried, ground tissues of whole marine pelagic fish like anchovy (*Engraulis* sp.), menhaden (*Brevoortia* sp.), capelin (*Mallotus villosus*), blue whiting (*Micromesistius poutassou*), sandeel (*Ammodytes* sp.) and Atlantic herring (*Clupea harengus*) (IFFO, 2005). It is estimated that feed costs comprise up to 60-70% of total production costs in intensive aquaculture where fish meal constitutes the most expensive part of the total dietary costs. Fish meal prices have increased as a result of global over fishing occurring simultaneously with increasing demand for feed raw materials as aquaculture grows. Furthermore, global fish meal production is vulnerable to environmental fluctuations (for ex. El Niño) making fish meal production and prices unpredictable.

Fish meal replacement is presently the subject of considerable research (Mundheim *et al.,* 2004; Espe et *al.,* 2006; Helland & Grisdale-Helland, 2006; Førde-Skjærvik, 2006) with the aim of choosing ingredients depending on their availability, suitability and market price. Feed companies attempt to use feed formulations mixing various proportions of raw materials to accomplish a complete feed that satisfies the nutritional requirement of the fish, and is able to sustain maximal growth and good feed efficiency at the lowest possible price. In the short-term, effort is focused on better utilisation of existing marine raw materials in terms of by-products, *i.e.* fishery by-catches and discards (constituting approximately 600.000 tonnes per year – about 20% of all catches and farmed fish in Norway; www.rubin.no), and improved fish

meal quality produced from non-food fishes (Hardy *et al.,* 2001). It is estimated however that these resources are insufficient to meet the demand by the feed industry in the long term. Fish feed ingredients must additionally satisfy the criteria set by national and international authorities.

3.6.1. Terrestrial plant proteins

Fish meal replacement with plant sources has been the target of many studies (Olli *et al.,* 1994, 1995; Bjerkeng *et al.,* 1997; Storebakken *et al.,* 1998, 2000; Carter & Hauler, 2000; Refstie *et al.,* 2001; Opstvedt *et al.,* 2003; Navneet *et al.,* 2006). Soy protein products are among the most studied and perhaps the best accepted fish meal replacers due to the steady supply of soybeans, its high protein content and reasonable price (Storebakken *et al.,* 2000). The possible use of wheat, corn gluten and lupine meal has also been demonstrated to some extent (Hardy, 1996; Hansen *et al.,* 2006).

Problems using plant based diets include an imbalanced amino acid profile, most contain various anti-nutritional factors, lack of long chain ω-3 polyunsaturated fatty acids, have a high fibre content and the plant products may contain gene modified organisms. They also appear to be less palatable than marine ingredients. Total fish meal replacement has not been successful especially for carnivorous species, and are known to cause reduced growth rate or/and a decreased feed efficiency where the magnitude depends on the protein source and level of replacement (Mundheim *et al.,* 2004). Total replacement of fish meal with soybean products is also reported to affect sensory quality, especially flavour in salmonids (Kaushik *et al.,* 1995).

3.6.2. Terrestrial animal by-products

Animal by products such as meat, bone and poultry by-product meal has been used in feed formulations for many decades. Significant amounts of meat, bone and blood

meal have successfully been used as fish meal replacement in the diet of rainbow trout, Mozambique tilapia, gilthead sea bream and juvenile grouper without effect on performance (Tacon & Jackson, 1985; Davies *et al.,* 1989; Robaina *et al.,* 1997; Millamena, 2002). The use of these ingredients has been however limited due to poor digestibility and quality variability. Animal by-product meals contain crude protein in the range of 45-55%, and often have high ash content because a high amount of the materials include bone and other non-muscle materials (NRC, 1993). Spray-dried blood meal is rich in protein (89%) but have low level of essential amino acids isoleucine and methionine (NRC, 1993). Feather meal is high in crude protein (80%), but has low digestibility if not thoroughly hydrolysed during processing (Cho & Slinger, 1979). Better manufacturing practices have however shown to increase digestibility of these ingredients (Bureau *et al.,* 1999).

Concerns about the possible transfer of animal infectious agents such as Bovine Spongiform Encehalopathy (BSE) and avian influenza has lead to increased consumer awareness of feed and food safety (SCAHAW, 2003). Allowed feeds raw materials from animal origin are strictly regulated and banned in fish feeds (EC 1774/2002 and 93/2005), but few exceptions exist: blood meal from non-ruminant animals is allowed in EU provided that is has been processed in accordance with conditions defined by regulations (EC 1292/2005). Despite the recent changes in EU legislation, European salmon manufacturers currently do not use feeds with animal by-products for fear of consumers' reactions, retail restrictions, and when waiting for new common international regulations for allowed ingredients (Huntington, 2004). Animal by-product meals may have promise as partial substitutes for fish meal, especially when modern handling and processing techniques have improved significantly the safety and quality of these meals (Bureau *et al.,* 1999).

3.6.3. Single cell products

Bacterial protein meal produced on natural gas is a new ingredient with reported similar proximal composition and amino acid profile as high-quality fish meal

(Skrede *et al.,* 1998). The availability of this raw material is still limited and the price is high. A study with salmon resulted in a minor linear decrease in crude protein digestibility (Skrede *et al.,* 1998), and slightly reduced growth rate (Berge *et al.,* 2005) when fish meal was replaced with bacterial protein. Similarly, there was a linear decrease in nitrogen absorption and an increase in the excretion of urea in rainbow trout with increasing bacterial protein level in the diet (Perera *et al.,* 1995). No significant effects have been observed on the sensory characteristics of salmon with regard to taste, smell or texture when up to 20% of fish meal was replaced with bacterial proteins (Berge *et al.,* 2005).

3.6.4. Alternative marine meals

Global production of discards in commercial fisheries has declined from approximately 27 M tonnes to 7 M tonnes from the early '90s (FAO, 2004b), where shrimp trawl fisheries estimated to constitute for up to one-third of the global total discards. Lack of marine feed ingrediets has resulted in increased utilisation of the discards with improved processing technologies and expanding market opportunities for them. The total utilisation of by-products in Norway was estimated to be approximately 457 000 tonnes, 73% of the total by-product production in 2005 where the herring constituted the largest amount of the marine by-products (www.rubin.no). Largest amount of the by-products were processed to meal for aquaculture.

Conserving of fish-by-products is one of the main challenges as high degree of hydrolysis of raw materials may create bitter taste peptides (Alder-Nissen, 1984) and together with lipid peroxidation may cause variability in raw materials. Ensiling raw materials in the formic acid has been developed to produce more predictable and reproducible silage product, called fish protein hydrolysate (Hevrøy, 2004). Currently, the Norwegian legislation does not allow the use of trimmings and processing wastes from aquaculture to aquaculture feed (national fish health statement). However, consider to allow "cross species feeding" - the use of farmed

salmon to cod and vice versa in Norway (O.M. Lømo, The Norwegian Seafood Federation, FHL, Norway, pers. comm.).

Crustacean by-products in terms of shrimp, crab and lobster meal are potential alternatives for use as feed ingredients. The advantage using waste from crustaceans is that they also contain a source of natural astaxanthin that can be used for fish pigmentation instead of using synthetic pigment sources. Crustacean by-products, shrimp and crab meals, often have low available protein content compared to high-quality fish meal, and crustacean by-products contain a high ash and chitin content that limit the use of these meals at high inclusion levels. The use of these raw materials in fish feeds is currently limited.

3.7. Effect of diet on product quality

Increasing worldwide aquaculture production has changed from the traditional quantitative production to focus more on the qualitative production where issues related to flesh quality and consumers' preferences are concerned. The composition of feed for farmed fish has been under continuous changes, and different raw materials are chosen after availability and price. It is important to study how different feed raw materials affect the chemical as well as sensory quality of fish. Factors that affect final product quality include both pre- and post slaughter conditions; *i.e.* husbandry, feed and nutrition, feeding regime, slaughter process, fish handling and storage (for review; Rasmussen, 2001).

3.7.1. Flesh chemical composition

The fatty acid composition of the diet is reflected in the lipid deposition in the tissue of Atlantic salmon (Waagbø *et al.,* 1991; Espe & Lie, 2001; Bell *et al.,* 2004; Torstensen *et al.,* 2004) so it is possible to manipulate the muscle fatty acid

triacylglycerol content via dietary means. High dietary plant oil level lowers the diet level of ω-3 PUFA and increases the ω-6 level in the muscle lipid tissue (Waagbø *et al.,* 1993; Thomassen & Røsjø, 1989; Bell *et al.,* 2004). Study of wild vs farmed fish (channel catfish, rainbow trout and coho salmon) showed higher ω-3 : ω-6 fatty acid ratio in wild fish, but up to 5 times higher total lipid content was observed in farmed fish providing approximately similar total ω-3 PUFA content in wild vs farmed fish (Nettleton, 2000). The high dietary ω-3 PUFA level is desirable in human consumption since it has beneficial effects on cardiac diseases, levels of triacylglyserols in plasma, blood pressure and inflammatory responses (Kris-Etherton *et al.,* 2003). Fish has also capacity to modify fatty acids to fit a narrower and more preferred composition (Olsen *et al.,* 1999).

The development of feed manufacture has led to the production of feeds of higher nutritional value with high energy levels. High energy diets have resulted in the decrease in a feed conversion ratio, where lipids are used for energy and deposited, and proteins are used for growth (Grisdale-Helland & Helland, 1997). High dietary lipid level increases the muscle lipid content as there is a linear relationship between dietary lipid content and whole body and fillet lipid level in salmonids (Lie *et al.,* 1988; Bell, 1998; Einen & Skrede, 1998; Regost *et al.,* 2001) and in halibut (Aksnes *et al.,* 1996; Nortvedt & Tuene, 1998). Muscle lipid level is also dependent on fish size, normally increasing with higher body size. Furthermore, genetic variation between strains has a great impact on lipid deposition in fish (Rye & Gjerde, 1996).

Dietary protein has less influence on body protein content compared to lipid (reviewed by Austreng & Krogdahl, 1987). No correlation between whole body protein and dietary protein level in rainbow trout diets was reported by Kim *et al.* (1991). Only small decrease in muscle protein content was found in rainbow trout when fed with fat-enriched diets (Alsted, 1991).

Unlike the salmonids and halibut, cod muscle is lean and muscle lipids seldom exceeds 1% of the fillet mass. Main lipids in cod muscle are structural phospholipids,

and hence difficult to change by dietary modification, as opposite to salmonids, where surplus energy is stored as triacylglyserols in muscles and viscera. Cod store surplus energy predominantly in the liver (Dos Santos *et al.,* 1993).

3.7.2. Fillet colour

Delicate pink/red colour of salmonids flesh is perhaps the most important quality criteria for the consumer (Torrissen; 1989; Moe, 1990; Sigurgisladottir *et al.,* 1997; Anderson, 2000; Robb, 2001a; Cardinal *et al.,* 2004). Pigment cannot be synthetised *de novo* (Schiedt, 1998) and in nature the pink colour of salmonids is achieved by ingesting crustaceans that contain natural carotenoids mainly bonded in the exoskeleton of these organisms. Deposition of astaxanthin occurs during the long period of feeding. Commercial salmon feeds are supplemented with synthetic pigment source, mainly astaxanthin, typically 50-70 mg kg^{-1}, and cost farmers up to 20% of feed costs and 6-8% of salmon total production costs (Torrissen, 1995; Sinnott, 2001; Johnston *et al.,* 2006). Since there is increased concern about synthetic additives in feeds and foods, organic farmed salmonids are coming in to markets as niche products. Possibly, different macrozooplankton species can substitute synthetic astaxanthin in salmonid farming in the future. Further knowledge is needed on the levels and sources of different zooplankton species as a natural pigment source for salmonids.

The flesh of cod and halibut are expected to be white, and even small colour deviations may result in rejection from the consumers. The coalfish and saithe fetches a poorer price than cod in some European markets that may be related to their greyer muscle colour compared with that of cod (Love, 1988). Replacing fish oil with 40% soybean oil in diets for farmed cod have been shown to reduce muscle lightness measured instrumentally as the *L**-value (Mørkøre, 2006). Fatty acid composition, and the presence of antioxidants in diets influences fatty acid oxidation and hence formation of oxidation substances (e.g. malonedialdehyde) that may affect muscle colour (Waagbø *et al.,* 1993; Ruff *et al.,* 2002).

Farmed cod has been shown to have lighter muscle colour than wild counterparts that may relate to higher muscle dry matter content in farmed fish and altered light reflection and reduced transparency (Robb, 2001b; Stien *et al.,* 2005), or lower level of dark muscle tissue (Grigorakis *et al.,* 2003). The effect of different protein sources on cod muscle colour is not well known, but altering dietary protein content between 36-66% has not resulted in any changes (Hemre *et al.,* 2004).

3.7.3. Skin colour

The skin colour of cod is of importance, as cod is sold whole or gutted in many markets. Farmed cod have generally darker skin than their wild counterparts, and darker colour is considered as lower quality characteristic in some markets (Mørkøre, 2005). The presence of carotenoids in the diet may alter the cod skin colour. Red colorisation of wild cod (*taretorsk*) is observed along the Norwegian cost due to high dietary levels of crustaceans containing astaxanthin (Love, 1974). Maize gluten meal in cod diets has also been reported to give more yellow hue in the cod skin, but no effect on the flesh colour has been seen (Ø. Karslen, IMR, Bergen, Norway, pers. comm.).

3.7.4. Sensory quality

Sensory evaluation is defined as "a scientific method used to evoke, measure, analyse, and interpret those responses perceived through the sense of sight, smell, touch, taste and hearing" (Stone & Sidel, 1993). Flavour of the fillet develops due to complex interaction of hydrolysis of proteins, oxidation of fatty acids and accumulation of volatile and non-volatile aroma compounds (alcohols, aldehydes and ketons and others), and miscellaneous compounds such as chloroform and trimethylamine (Grigorakis *et al.,* 2003). Only humans can perceive, describe and

quantify sensory characteristics important to the consumers. Sensory tests are often performed using a trained panel according to the International Standard 8586-1 (ISO 1993), but these assessments are time consuming and expensive. Instrumental quality studies, quality index method and chemical analysis in addition to sensory analysis have been successfully used in later years for quality assessments (FAO, 1995; Stien *et al.,* 2006).

Several authors have investigated the influence of dietary fatty acid composition on the sensory quality of fish (Hardy, 1987; Thomassen & Røsjø, 1989; Skonberg *et al.,* 1993, Waagbø *et al.,* 1993). Muscle lipid level and fatty acid composition, and the presence of antioxidants affects the rate and level of oxidation, and hence formation of compounds that have organoleptic impact on the final product (Waagbø *et al.,* 1993).

Generally, different protein sources in salmonids diets have shown only little effect on quality of salmonids (Bjerkeng *et al.,* 1997). Kaushik *et al.* (1995) reported altered sensory quality, especially flavour, when salmonids were fed with soybean products. Increasing dietary lipid and hence muscle lipid deposition is reported to have impact on sensory juiciness in salmonids (Johansson *et al.,* 1991; Einen & Thomassen 1998). However, sensory muscle quality in cod is more difficult to change by dietary modification; altering dietary lipid level between 6-30% was reported not to have any effect on sensory attributes in cod (Hemre *et al.,* 2004; Mørkøre, 2005).

Impact of marine feed raw materials on the organoleptic studies is a few. Sipos & Ackman (1964) showed that large amount of ingested marine invertebrates resulted in blackberry flavour of Atlantic cod. Enhanced sensory quality was reported in rainbow trout fed diet supplemented with krill (*E. pacifica*) (Spinelli *et al.,* 1979). Zooplankton contains high level of free amino acids where proline may sweeten the flesh, and anserine is described as “satisfactory for mouth” (Love, 1988; Espe & Lie, 2001). Inclusion of crab meal in cod diet has been shown to camouflage the negative taste and odour when using lupine meal as fish meal replacement (S. Albrektsen,

Fiskeriforskning, Bergen, Norway, pers. comm.). More knowledge is needed to document the effects of different macrozooplankton meals on product quality in Norwegian common cultured Atlantic salmon, halibut and cod.

3.7.5. Muscle texture

Texture is defined as the response of the tactile senses to physical stimuli that result from contact between some part of the body and the food (Bourne, 1982). Muscle firmness is one of the most important flesh quality traits in fish (Sigurgisladottir *et al.,* 1997) and influences various attributes including chewiness, dryness, moisture and mouth feel (Haard, 1992). Instrumental testing of texture has been showed to correlate well with sensory texture analysis (Mørkøre, 2002).

Various factors are reported to affect texture properties in fish including muscle fibre size (Johnston *et al.,* 2000), collagen content (Sato *et al.,* 1986), fat content (Johansson *et al.,* 2000), pH (Dujanski, 1979), starvation (Einen & Thomassen, 1998) and storage time (Færgemand *et al.,* 1995). Textural properties are also reported to differ between farmed and wild fish (Johnston *et al.,* 2006). There is lack of knowledge on how different crustacean meals affect *post mortem* muscle texture. Spinelli *et al.* (1979) reported enhanced texture in rainbow trout fed with krill (*E. pacifica*). Sensory quality of cod was not affected varying the dietary level of protein (36-66%), lipid (9-27%) or starch (6-18%), in the diet (Hemre *et al.,* 2004). More knowledge is needed to document if different zooplankton species have any effect on *post mortem* texture in fish.

4. General discussion

4.1. Methodological considerations

4.1.1. Raw materials and diets

Macrozooplankton (*T. libellula* and *T. inermis*) were caught during scientific surveys in the south-western part of the Norwegian Sea, close to Jan Mayen, by pelagic trawl by the Institute of Marine Research vessel, G.O. Sars, in June 2002 and May 2003, respectively. Catches constituted from single schools and were immediately block frozen on board at –20 ºC to prevent degradation. Antarctic krill was purchased block frozen from Superba Invest, Ltd, Ålesund, Norway. Meals that were used in **Papers I-V** are specified in Table 4.

Table 4. *Source of meals that were studied in* ***Papers I-V****.*

Paper	Fish meal (Control)	*T. inermis*	*T. libellula*	*E. superba*
I	X			X
II	X	X	X	X
III	X	X	X	X
IV	X			X
V	X	X	X	X

Macrozooplankton whole meal was processed similarly to the traditional fish meal production line at the pilot plant of the Norwegian Institute of Fisheries and Aquaculture Research, Fyllingsdalen, Norway. Budzinski *et al.* (1985) have described this type of process in detail for Antarctic krill. Briefly, diets were prepared from frozen raw materials followed by thawing, heating to about 90 ºC (scraped

surface heat exchanger (Alfa Laval), and pressing in a screw press (Figure 4). The press liquid was then run through a separator to separate liquid from the oil. Liquid was concentrated by evaporation and added to the presscake. Antioxidant (mixture of ethoxyquin and formic acid) were added and the mixture was dried in a hot air dryer to produce meal. The final diets were cooked by co-rotating twin-screw pilot scale extruder (Wenger TX-52, Sabetha, USA), dried in hot air (Paul Klöckner, type 200.2 carousel dryer, Nistertal, Germany), cooled and finally the diets were coated with the fish-oil (Norsalmoil) in a vacuum oil coater. The experimental diet production is described in detail in **Paper I** and **III**.

Fish meal and meals prepared from macrozooplankton in this thesis had a low level of biogenic amines (**Papers I** and **III**). As these are produced by enzymatic processes during degradation of the raw material, (Pike, 1993; Aksnes & Mundheim, 1997) it was interpreted that the quality of the meals were high. Water-soluble protein fraction showed slightly higher values for krill meal compared to other meal sources, and may reflect the high proteolysis found in krill than found in amphipod (*T. libellula*) and fish meal (Langmyhr & Mejde, 2003; **Paper III**).

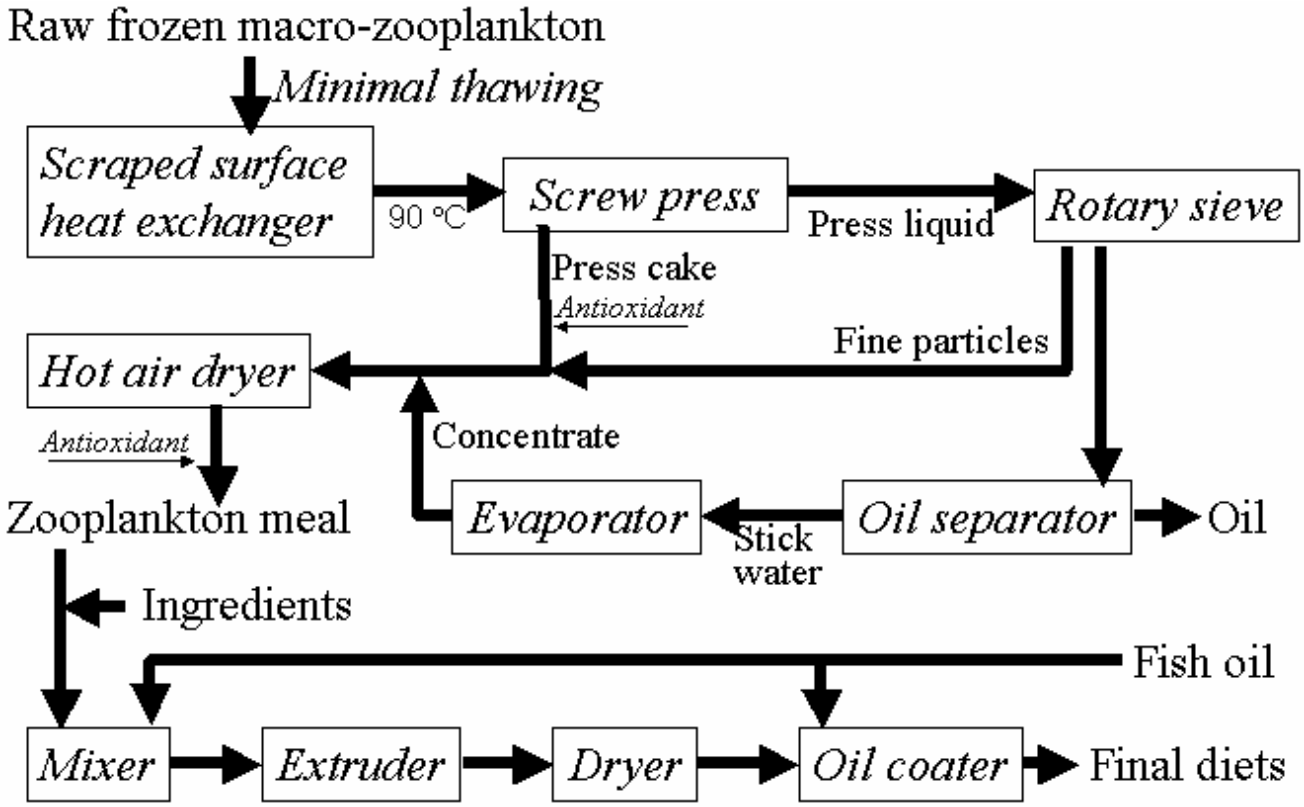


Figure 4. *Macrozooplankton feed preparation*

The major difference between meals prepared from macrozooplankton and fish meal (approximately 93% dm), was the lower level of crude protein 524-641 g kg^{-1} in krill meal compared to fish meal 732 g kg^{-1}. One main reason for the lower protein content in macrozooplankton meal appeared to be the higher ash content that originates from the exoskeleton of crustaceans. The ash content varied being lowest in fish meal and northern krill meal (124 g kg^{-1} and 104 g kg^{-1}, respectively) and highest in amphipod meal (222 g kg^{-1}). Macrozooplankton was also higher in salt content (NaCl) 38-59 g kg^{-1} compared to fish meal 23 g kg^{-1}. This was due to the higher level of collected seawater with the krill compared to the fish. The amount of chitin that originated from the exoskeleton of macrozooplankton showed high variation between species; it was particularly thick in amphipods, and meal prepared from the them (*T. libellula*) resulted in the highest chitin content (100 g kg^{-1}) compared to northern krill (*T. inermis*) (30 g kg^{-1}) and Antarctic krill (*E. superba*)

(40 g kg^{-1}) meal, while it was absent in fish meal. The meal prepared from northern krill differed from fish-, Antarctic krill- and Amphipod-meals in that it contains the highest level of lipids (182 g kg^{-1}), about twice of that in the other sources (75-99 g kg^{-1}).

Whole meals prepared from krill also contained a high level of natural astaxanthin where approximately 70% were in the form of astaxanthin esters. The total astaxanthin level found in Antarctic krill and northern krill meal were 99 and 114 mg kg^{-1} dm, respectively (**Paper III**), which was within the same range as reported earlier (Kotik *et al.,* 1979; Quantz, 1980; Yamaguchi *et al.,* 1983 and Foss, 1985; reviewed by Storebakken, 1988). Meal prepared from the amphipod *T. libellula* was low in astaxanthin (10 mg kg^{-1} dm) (**Paper III**).

4.1.2. Diet properties and experimental design

During feed production, increasing fish meal substitution with macrozooplankton meal gave less expansion during the extruding process than using standard fish meal diets. High level of water-soluble protein (approximately 50%) in macrozooplankton meal affected the pellet properties and expansion preventing efficient lipid adsorption of the pellets during vacuum coating. This forced us to add higher level of lipid into the mash before extrusion to reach the desired total lipid content of approximately 30% in final diets (**Paper III**):

- 13% lipid into mash (dm) for diets including 0-20% macrozooplankton meal
- 14% lipid into mash (dm) for diets including 40% macrozooplankton meal
- 15% lipid into mash (dm) for diets including 60% macrozooplankton meal

Using this procedure, the diets contained sufficient amounts of lipids, but the pellets were slightly softer and had lower water stability (**Paper I**). This did not affected fish feeding in our experiments, but feed collectors had to be checked more

frequently to prevent the pellets dissolving in the feed collectors. Under practical conditions, use of macrozooplankton may limit the preparation of high-energy diets that contain more than 350 g kg^{-1} of lipids.

Due to lower crude protein level in macrozooplankton meals, higher levels of them had to be included into the diets than fish meal in order to reach the same protein level in the complete diets. By altering the diet carbohydrate level (5-14%), the diet volume could be regulated without affecting the diet energy content to any major extent. Diet energy content varied between 23 and 24 MJ kg^{-1} (**Paper III**). Macrozooplankton meal also contributed to diet total lipid content to some extent. Hence, lower level of fish oil had to be added to diets prepared from macrozooplankton to reach the same total lipid content as using fish meal.

The fatty acid profile of experimental diets remained mostly unaffected when macrozooplankton meals were used as fish meal replacement as most of the lipid was added to the diets in the form of fish oil. There was however a trend for higher dietary 20:5n-3 level and lower level of monounsaturated fatty acids as the amount of krill meal was increased, and a higher monounsaturated fatty acid 20:1n-9 content using amphipod meal (**Paper III**). Higher dietary monounsaturated fatty acid and ω-3 PUFA level is also reported by Floreto *et al.* (2001) and Opstad *et al.* (2006) when dietary level of zooplankton was included in the diet.

Replacing fish meal with macrozooplankton for up to 60% of diet proteins did not affect diet essential amino acid content to any major extent. Amino acids histidine, methionine and lysine showed small decreases as fish meal was replaced with meal from northern krill (*T. inermis*) while levels of arginine, isoleucine and phenylalanine increased. Similarly, Floreto *et al.* (2001) reported higher dietary arginine values when fish meal was gradually replaced with freeze-dried krill hydrolysate. According to NRC (1993) all diets held levels of essential amino acids above described requirements for optimal growth.

Table 5. *The level (% of diet protein) and source of zooplankton that replaced fish meal in several fish species in* ***Papers I-V****.*

Paper	Fish meal (Control)	*T. inermis*	*T. libellula*	*E. superba*	Fish species
I	X	-	-	0-100%	Atlantic salmon
II	X	0-60%	40%	40%	Atlantic salmon
III	X	0-60%	40%	40%	Atlantic salmon/halibut
IV	X	-	-	0-100%	Wild cod/farmed cod
V	X	-	-	0-100%	Farmed cod
V	X	40%	40%	40%	Atlantic salmon

4.2.1. Recording of growth

In the present studies fish were collected and sorted at the initial of feeding experiments to be of equal size in different tanks among replicates. Additionally, tank order was randomised with regard to diet in order to control and minimise the environmental influence on feeding and appetite. Atlantic salmon were individually tagged by T-Bar anchor tags (Floy Tag & MFG. Inc., Seattle, WA, USA) where the fish weight and length was measured in individual fish from duplicate or triplicate tanks in **Papers I** and **III**, respectively. Atlantic salmon biomass in the tanks were kept under 30 kg per m^3, as higher biomass in these tanks (above 35 kg per m^3) may reduce weight gain and feed intake (R.E. Olsen, pers. comm.). Hence, the amount of fish was reduced in tanks when the first sampling took place. Weight and length of halibut in **Paper III** and cod in **Papers IV** and **V** were measured in duplicate tanks including 40-50 fish per tank. Halibut skin was photographed at the beginning of the trial, after 78 days and 150 days of feeding (**Paper III**). The intention was to individually recognize the fish between sampling and hence get individual growth data of these fishes. However, the skin colour of halibuts changed during feeding periods and made it too difficult to find individuals between sampling. The weight and length measurements in halibut and cod were based on mean values of fish in duplicate tanks.

Specific growth rate (SGR) in all fish was calculated according to the formula:

$$SGR = 100 \times (\ln(W_{end}) - \ln(W_{start}) / \Delta t) \qquad [eq.\ 1]$$

where W_{end} and W_{start} denotes end and start weight of fish and Δt experimental days. The fish growth is proportional to the size of the animal being higher in small compared to larger animal (Brett, 1979).

4.2.2. Digestibility assessment

The apparent digestibility (AD) of macro-nutrients were studied (**Papers I**; **III**) incorporating yttrium oxide (0.1%) as non-toxic inert marker in the diet. Yttrium oxide was quantified in faeces after collecting faeces from the distal part of the intestine by stripping (Ringø, 1991) (**Paper I** and halibut in **Paper III**) and by dissection the intestine (Atlantic salmon in **Paper III**). Digestibility studies are more challenging in fish in water environment compared to terrestrial animals. Also, the methods how the faeces are collected from fish may affect deviations in results between studies (Windell *et al.,* 1978; Hevrøy *et al.,* 2005). The dissection and stripping procedures represent a risk for underestimation of digestibility by contamination with endogenous material.

Different inert markers, traditionally chrome oxide (Edin, 1918), but recently oxides of yttrium and lanthanides have been evaluated and found acceptable as inert markers in salmonids and cod digestibility studies (Austreng *et al.,* 2000; Otterå *et al.,* 2002). Relation between inert marker and nutrients present in faeces and feed can be compared and apparent digestibility coefficient (ADC) calculated using equation:

$$ADC = 100 - 100 \times ((Y_{feed} / Y_{faeces}) \times (N_{faeces} / N_{feed})) \qquad [eq.\ 2]$$

where Y_{feed} = yttrium oxide in feed, Y_{faeces} = yttrium in faeces, N_{faeces} = nutrient in faeces, N_{feed} = nutrient in feed. This method is called "apparent" digestibility since it does not take consideration the endogenous nutrients that origins from the fish body.

4.2.3. Feed conversion studies

The fish were fed to satiation with all experimental diets using disc feeders adjusted approximately 10% overfeeding. Feed collectors were used to to estimate the amount of uneaten feed and sampled at mornings before next feeding. Feed conversion ratio (FCR) was estimated in Atlantic salmon (**Papers I**; **III**), halibut (**Paper III**) and cod (**Papers V**) using formula:

$$FCR = \Delta \text{ feed} / \Delta \text{ growth} \qquad \text{[eq. 3]}$$

where Δ feed is the amount of feed in grams consumed by the fish in the tank between each weighing of the fish, and Δ growth is the increase of fish weight in grams (biomass in the tank) during the same period.

4.2.4. Muscle pH and rigor development

Post slaughter muscle pH was measured from white muscle tissue and used to evaluate the glycolytic- and rigor status in fish fed different protein sources (**Paper II**). Rigor development was measured as changes in muscle fillet hardness and measured by texture analyser as described by Kiessling *et al.* (2006). Both nutritional and pre- and post slaughter conditions are known to affect the development of muscle pH and rigor development (Stien *et al.,* 2005; Kiessling *et al.,* 2006). Low post

slaughter pH is associated with faster rigor development (Stien *et al.,* 2005) and may cause partial separation of muscle segments called gaping (Lavety *et al.,* 1988). This phenomenon has of considerably economic importance to the fish industry as gaping decreases the technological and market value of fish (Dujanski, 1979). Muscle pH was also studied in wild cod and compared to farmed cod fed fish meal control diet or with Antarctic krill diet (**Paper IV**).

4.2.5. Fillet colour and skin colour

Atlantic salmon muscle colour was studied both visually using Salmofan™ (F. Hoffmann-La Roche Ltd, Basel, Switzerland) and instrumentally using tristimulus colorimeter-HunterLab. Visual colour evaluation was performed comparing Atlantic salmon flesh colour to the series of numbered colour scale (20-34) of Salmofan™. This muscle colour measuring method is perhaps the most widespread today. The results of Salmofan™ measurements agreed with the results obtained by instrumental colour measurements. Visual colour method was however excluded from the **Paper II**, as the colour evaluation is very subjective method and should be performed in standardised lightness and trained and preferably same personnel between measurements. Instrumental colour measurement was compared to chemically quantified astaxanthin content (**Paper II**). Earlier studies have shown that the output colour values from tristimulus colorimeters correlate well to chemically quantified astaxanthin content (Wathne *et al.,* 1998; Robb, 2001a).

In **Paper IV**, the skin and muscle colour of wild and farmed cod was measured using tristimulus colorimeter. Both cod skin and muscle colour were measured at three points above and under lateral line and the mean values of these measurements were used.

4.2.6. Instrumental muscle texture analysis

Atlantic salmon (**Paper II**) and cod (**Paper IV**) muscle fillet hardness were studied (56 h *post mortem*) using the TA.XT2 (Stable Micro System, Surrey, England) texture analyzer, equipped with a calibration cell load of 5 kg and the software Texture Expert version 1.0 *SMS* for PC (Stable Micro Systems). Analyses were performed using flat-ended plastic cylinder probe (12.5 mm diameter) pressing it at 80% of the fillet thickness as it has shown to correlate well with sensory hardness evaluations (Mørkøre, 2002). Five measurements from caudal to the dorsal of the fillets were measured.

4.2.7. Chitin analysis

One of the study objectives was to develop a method to measure the level of exoskeleton chitin in the experimental diets and in faeces. The first method that was tested was obtained from Yabe *et al.* (1996) where these authors determined the chitin content in the yeast cell wall. Method described briefly included sample feed sample hydrolysis, amine colour reaction with *p*-dimethylaminobenzaldehyde and colorimetric detection with a spectrophotometer. This method was tested with the diets where were 0-100% of fish meal was gradually replaced with Antarctic krill meal (**Paper I**). Increased colour reaction was achieved with this method, but reagent *p*-dimethylaminobenzaldehyde probably reacted, in addition to dietary chitin, with other amino acids since the colour intensity was much higher as expected. This analysis was not tested further.

Another method that was tested and developed further was obtained by Rungruangsak-Torrissen & Sundby (2000) measuring free amino acids and nitrogen-containing compounds in the plasma. This method was used to measure acid-hydrolysed proteins (amino acids) and chitin (*D*-glucosamine residues) in experimental diets and faeces separated by high- performance liquid chromatography (HPLC) (**Paper I**). Peaks obtained from HPLC were then identified via comparison with the reference of known amino acid standard (Sigma-Aldrich Ltd, Steinheim,

Germany) and *N*-acetyl-glucosamine standard. Disadvantage using this method was, that the sample hydrolysis took for 22h and sample separation in HPLC for 120 minutes for one sample. Additionally, the chitin present in diets and faeces samples was unequally distributed in sample matrix, as the exoskeleton pieces in the diets were as small flakes after milling the raw materials. This caused variations in the amount of "exoskeleton pieces" between parallel samples. Higher amount of replicates were analysed (n= 5).

Analysing faeces appeared to be more challenging than analysing the feed. Faeces samples that originated from control fish fed fish meal diet devoid of chitin gave a peak for chitin (*N*-acetyl-glucosamine), simultaneously with the standard sample containing *D*-acetyl-glucosamine. Investigating further, it became clear that intestinal mucus contained both *D*-acetyl-glucosamine and *N*–acetyl-galactosamine where the former had to be reduced from the "total amount of chitin". This was done measuring the relation of *N* –acetyl-glucosamine and *N* –acetyl-galactosamine in faeces in intestinal mucus samples that was found 15.4 : 1. Intestinal "real" amount of chitin could be then calculated from the relation of these two amino sugars (**Paper I**). However, the amount of intestinal mucus may vary in fish fed different fish depending on the diet making this method unreliable. Both halibut and cod showed that chitin was highly indigested. These results were excluded from the studies however, because of the high variation between results in parallel analysis.

4.3. Comparative feed conversion and growth of adult fishes fed macrozooplankton diets

Inclusion of macrozooplankton for up to 60% of diet protein did not significantly affect the feed conversion ratio (FCR) in salmon or halibut despite that these diets also increased the level of indigestible ash and chitin as the level of zooplankton increased (**Paper III**). Higher FCR was particularly noticeable in the experiment where fish meal replacement gradually increased from 0 to 100% with Antarctic krill (**Paper I**). Fish seemed to compensate for the higher level of indigestible matter by

eating more, and were able to maintain good growth in all experimental groups. Salmon compensatory growth at high dietary indigestible carbohydrate level has also been reported previously by Hemre *et al.* (1995) and Sveier *et al.* (1999). Atlantic salmon and halibut seemed to accept more readily diets where fish meal was replaced with macrozooplankton meal. This was most pronounced at the beginning of experiments and lasted for up to 100 days of feeding (**Papers I**; **III**). Zooplankton contain a high proportion of (6.6-9.5% dm) free amino acids (Ellingsen, 1982) that may act as a chemoattractants diffusing free amino acids into the surrounding water and enhancing the palatability of feed as suggested by Pike *et al.* (1990); Holm & Walther (1988); Kolkovski, (2000); Floreto *et al.* (2001). The palatability of feed is of primary importance as 70-80% of measured growth variation can be explained by differences in feed intake (Rosenlund *et al.,* 2005).

Fish meal replacement with northern krill (*T. inermis*) in diets significantly stimulated the specific growth rate (SGR) both in Atlantic salmon and halibut (**Paper III**); Atlantic salmon significantly showed higher SGR during the first 100 days of feeding, with 0.92 vs 0.87 in highest inclusion of northern krill and control group, respectively (**Paper III**). Halibut showed the same enhancement in SGR with northern krill during the whole 150 days experimental period, with 0.39 vs 0.33 in the highest northern krill inclusion and control group, respectively (Figure 5; **Paper III**). Low SGR has been a major challenge and restricting factor in halibut farming in Norway (T. Harboe, pers. comm.). By adding krill meal to the halibut diets growth rate can be increased significantly.

Addition of amphipod (*T. libellula*) meal in the diet, on the other hand, performed differently than northern krill; no growth enhancement in halibut were seen when fish meal was replaced with amphipod meal up to 40% of diet protein. However, significantly enhanced SGR (0.85 vs 0.80) was seen in Atlantic salmon during the whole 160 days experimental period, when fish was replaced with amphipod meal for up to 40% of diet proteins (Figure 6). Moreover, the inclusion of northern krill and Antarctic krill in the salmon diet (40% of diet proteins) produced a

trend for higher growth during the whole experimental period when compared to the control fish meal diet (Figure 6).

The reason for the differences in growth responses between salmon and halibut fed similar diets may be that they respond differently to the feed attractants (Mackie & Mitchell, 1982). Higher growth rate especially seen at the first feeding period in Atlantic salmon and halibut indicated stimulated feed intake from the initial of the experiments. This growth enhancement lasted throughout the study, even though the differences were smaller during the later feeding period from 78/100 days until 150/160 days of feeding in Atlantic salmon and halibut, respectively (**Papers I**; **III**). Krill extract 2% in the diet (Nosan, Yokohama, Japan) has also showed to enhance the digestive capability in yellowtail (*Seriola quinqueradiata*) (Kofuji *et al.,* 2006) that gives an alternative explanation for enhanced growth seen in salmon and halibut fed moderate amount of zooplankton in our studies. Trypsin activity and different trypsin isozymes have been postulated to limit the growth rate in cod and Atlantic salmon (Lemieux *et al.,* 1999; Rungruangsak-Torrissen *et al.,* 1999).

Krill supplementation (5%, combination of dried and frozen; Inual/Tepual, Santiago, Chile) has also been shown to enhance the diet palatability when using soybean meal as fish meal replacement for Coho Salmon (*Oncorhynchus kisutch*) (Arndt *et al.,* 1999). Furthermore, substitution of soybean meal with freeze-dried krill hydrolysate 75 and 100% of the dietary protein increased the body weight gain and survival of juvenile American lobster, *Homarus americanus* (Floreto *et al.,* 2001). Allahpichay & Shimizu (1984a; 1984b) reported that the growth promoting factors were located in the cephalothorax region of Antarctic krill since growth enhancement was succeeding when non-muscle krill meal was used instead of pure krill tail meat. They further investigated the growth promoting factors from Antarctic krill meal and concluded that these factors were steroids located in the non-muscle Antarctic krill meal (Allahpichay & Shimizu, 1985). More knowledge is needed on chemical composition, growth promoting factors and attractants present in zooplankton.

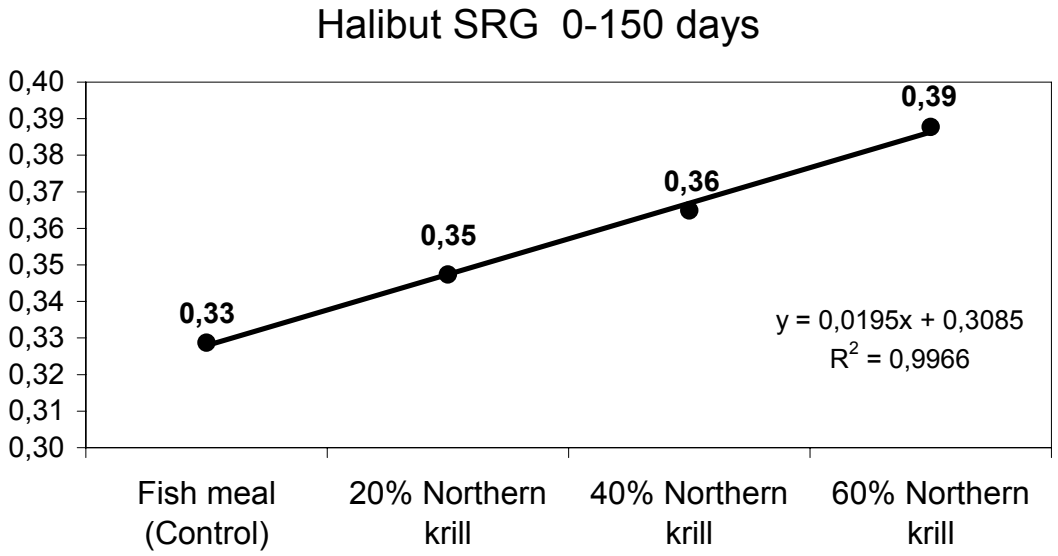


Figure 5. *Average daily growth in Atlantic halibut fed increasing amount of northern krill in the diet, ranging from 0-60% of the dietary protein.*

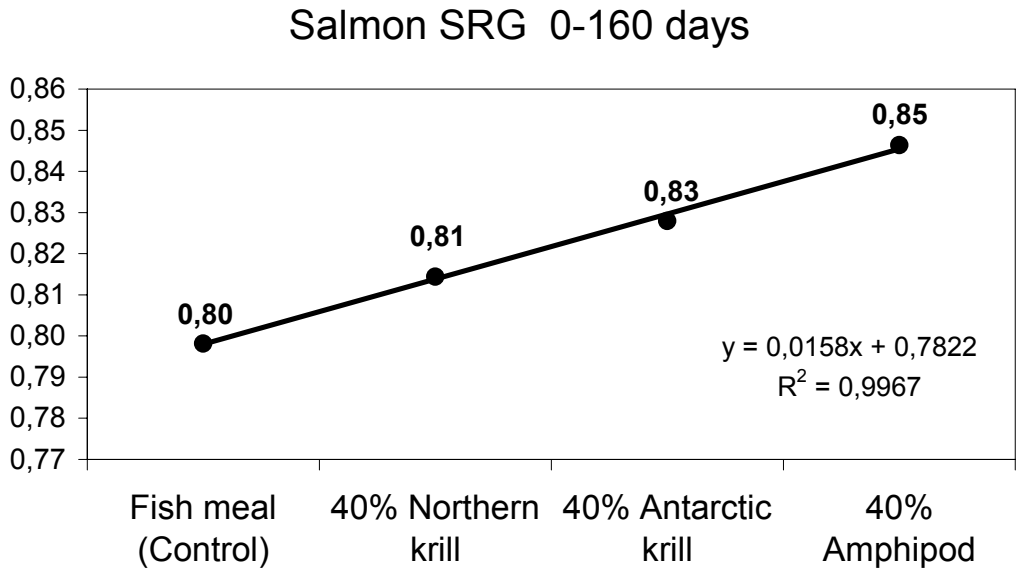


Figure 6. *Average daily growth in Atlantic salmon fed three types of macrozooplankton in the diet, at a level of 40% of diet protein.*

We were unable to detect any effect on growth in Atlantic cod (120-250 g) that were fed with Antarctic krill for up to 100% of diet proteins for 75 days. It may take longer for cod to reach significant differences between experimental groups. Similar

sized Atlantic salmon fed 50% of diet proteins from northern krill (*M. norvegica*) showed similar results, where no growth enhancement or feed conversion rate was seen (Ringø *et al.,* 2006). Furthermore, no adverse effects could be observed in growth pattern in any of the feeding studies in this thesis independent of inclusion levels of macrozooplankton or fish species used. Similarly to SGR, the k-faktor was stimulated in fish for up to 60% protein replacement level in both salmon and halibut indicating that fishes receiving zooplankton in the diets were generally heavier than fish fed without zooplankton (**Paper III**). That was most pronounced when Atlantic salmon were fed with an amphipod diet and halibut with a northern krill (*T. inermis*) diet.

4.4. Nutrient digestibility of macrozooplankton diets

Apparent digestibility (AD) of protein was similar in all experimental groups of Atlantic salmon, independent of protein source. In **Paper I**, protein AD varied between 87 and 89% regardless of the level at which fish meal was replaced with Antarctic krill meal (0-100%). In **Paper III**, protein AD ranged between 84 and 86% in all experimental groups up to 60% fish meal replacement with macrozooplankton. These results are in agreement with the earlier study where protein digestibility of Antarctic krill meal (including exoskeleton) in rainbow trout diet was 87% (Vens-Cappell & Horstmann, 1978). Atlantic halibut showed somewhat lower protein AD ranging from 74 to 79% from the lowest to highest inclusion of northern krill (**Paper III**). Different fish species seem to have adapted to digest different amounts of nutritional compounds. Also proteolytic enzymes may develop with fish size, and work more effectively in large salmon than small halibut (**Paper III**). Apparent digestibility studies in this thesis were performed in fish after approximately 5 months of feeding. It may take at least 78 days to fish to adapt for new environment and to adapt dietary system to dietary substrates as seen for halibut in **Paper III** that increased feed intake and SGR after 78 days of feeding. It is hence recommended that

digestibility studies should be performed in fish that is adapted in new environments and shows normal species SGR and feeding rate.

Lipid AD decreased from 93-95% to 90% in salmon when more than 80% of diet proteins were replaced with Antarctic krill meal (**Paper I**). The underlying reason for that may be the increased chitin content in the diets. Chitin may bind lipid and bile salts, and result in lower lipid hydrolysis by lipase and lower lipid absorption as have been shown in rainbow trout (Lindsay *et al.,* 1984) and tilapia *Oreochromis niloticus* x *Oreochromis aureus* (Shiau & Yu, 1999). High faecal chitin content may also explain higher moisture content in faeces and the diarrhoea-like condition with faster movement of digesta through the gastrointestinal tract observed with the highest degree of inclusion of Antarctic krill in the diet (**Paper I**). In **Paper III** somewhat contradictory results were obtained where the lowest faecal moisture content was found in fish fed with amphipod diet. This diet also included the highest level of indigestible chitin and resulted in the best growth of all dietary groups. Lipid AD in the Atlantic salmon was similar among the dietary groups in **Paper III**, but was inexplicably high in all experimental groups (98-99%): This might be the result of incomplete lipid extraction of the faecal matter with methanol-choloroform.

Chitin in moderate levels may have some advantageous functions in the digestive tract functioning as prebiotic, selecting autochthonous bacteria and inhibiting the growth and colonisation of pathogenic bacteria referred as immunostimulant (Sakai, 1999; Esteban, 2000; 2001). There were indications that the number of autochthonous bacteria increased 100 fold in the gut of Atlantic salmon when fed with northern krill (*M. norvegica*) (Ringø *et al.,* 2006). Salmon fed with the highest level of chitin originating from amphipods also resulted in significantly higher growth compared to the control group. Chitin levels of between 2 and 5% also resulted in higher growth in shrimp (*P. monodon*) when compared to diets without chitin inclusion (Shiau & Yu, 1998). Further studies of chitin in fish diets and the effects of chitin on fish health parameters are presently under way. Chitin digestibility was low in Atlantic salmon (**Paper I**), but showed a somewhat linear

increase (0-40%) from control fish meal diet towards total fish meal replacement with Antarctic krill meal.

Dry matter digestibility was high and stable in all groups and varied between 94 and 97% in salmon and halibut. Hatlen *et al.* (2005) reported significantly lower dry matter AD in Atlantic halibut ranging between 55.2 and 65.6%. Lower outcomes in that study may reflect the different fish size used. Alternatively, different moisture content in faecal material may have resulted in different values between our study and that of Hatlen *et al.* (2005). Stripping of faeces (Hevrøy *et al.,* 2005) or the faeces collecting technique (Choubert *et al.* (1979) have been reported to affect deviations in faecal moisture content.

4.5. Muscle quality

The muscle fatty acid composition was not affected to any major extent by replacing fish meal with macrozooplankton meals. The total muscle fatty acid composition was similar in all experimental groups of Atlantic salmon and halibut (**Paper III**). Diets prepared from northern krill contained a higher level of other lipids than that found in fish meal. This was seen as a tendency for higher muscle accumulation of monounsaturated fatty acids and 20:5n-3 fatty acids in Atlantic salmon fed diets including the highest level of northern krill meal (**Paper III**). Small Atlantic halibut muscle contained high PUFA levels (>50%) (**Paper III**). This probably reflects the lower lipid depots in smaller fish where the majority of the fatty acids are structural lipids (Greene & Selivonchick, 1990).

Based on the fatty acid composition found in salmon and halibut in **Paper III**, it can be concluded that macrozooplankton meal can replace fish meal, and maintain or even improve the marine fatty acid profile in the fish muscle. Even a study, where fish oil was totally replaced with lipids originating from zooplankton (copepod *Calanus*) in Atlantic salmon diets did not negatively affect muscle ω-3 PUFA fatty acid level (Olsen *et al.,* 2004). Marine ω-3 PUFA fatty acids are known to play a

beneficial role in several aspects of human health regarding cardiac diseases (e.g. Harris *et al.,* 2003), and when evaluating new feed raw materials it is important that these healthy marine fatty acids are maintained in fish muscle.

Muscle gross composition was unaffected regardless of the protein source and the level at which fish meal was replaced with macrozooplankton. Small differences were seen between two studies in the lipid and dry matter content in Atlantic salmon. The first study showed an approximate lipid and dry matter content of 208 and 284 g kg^{-1} respectively, in salmon fed Antarctic krill (**Paper I**). The latter study using three types of macrozooplankton gave an average muscle lipid and dry matter content of 272 g kg^{-1} and 328 g kg^{-1}respectively, in similar sized fish (**Paper III**). Dry matter was related to muscle lipid content in agreement with results by Hemre *et al.* (1992). Small Atlantic halibut had an approximate lipid muscle lipid content of 48 g kg^{-1} and dry matter content of 215 g kg^{-1} (**Paper III**).

Paper II included a pigmentation study where synthetic astaxanthin was compared to astaxanthin that origined from three different macrozooplankton species. Salmonids are incapable of synthesizing carotenoids and in nature the pink colour of salmonids is achieved by ingesting crustaceans. Total carotenoid levels in the diets were regulated to a minimum of 60 mg kg^{-1} in all diets. The outcome of this experiment showed that the salmon muscle astaxanthin level did not decrease when the level of synthetic carotenoid (Carophyll Pink®) decreased from 60 to 8 mg kg^{-1} in the diet while astaxanthin esters from zooplankton increased from 0 to 51 mg kg^{-1}, as the level of northern krill meal increased in the diet (control diet and 20, 40, 60% *T. libellula* meal inclusion). Based on this study it can be concluded that the astaxanthin esters in krill is utilised to a similar extent than that of free astaxanthin supplied as Carophyll Pink®. Thus, using these meals will reduce the requirement for synthetic astaxanthin needed in order to reach similar muscle pigmentation.

Technical muscle quality was assessed in Atlantic salmon fillets measuring fillet hardness with a texture analyser three day*s post mortem*. Fish meal replacement with three different macrozooplankton meals did not cause any major changes in

textural properties (**Paper II**). It was however obvious that the experimental group where 20% of fish meal was replaced with northern krill and the group where 40% of fish meal was replaced with amphipod meal displayed lower pH immediately after slaughter, showed faster rigor development and the highest fillet hardness 3 days after slaughter. These two fish groups also displayed the biggest fish size (not significant) used in this study, and the small differences seen in muscle hardness and onset of *rigor mortis* between treatments may be the result of differences in the growth rate rather than the source of protein, as growth rate is reported to affect tissue hardness (Einen *et al.,* 1999; Robb 2001b). Our results are in agreement with Hemre *et al.* (2004) who showed that macro-nutrient composition did not affect muscle texture *post mortem*.

Muscle texture of wild cod was softer and had lower total and maximum force values compared to farmed cod, when measured with a texture analyser as resistance towards compression in fillets (**Paper IV**). Farmed cod also showed lower *post mortem* pH 3 days after slaughter compared to wild cod (6.4 vs 7.0, respectively). These values were in agreement with earlier reports for pH in farmed cod by Stien *et al.* (2005) and Otterå *et al.* (2006). Higher *post mortem* pH seen in wild cod compared to farmed cod may reflect the lower feed intake and reduced muscle glycogen content as have been seen in starved cod (Love, 1988).

Analysis by a sensory panel was included in the study to investigate the sensoric acceptability of Atlantic salmon fed three different macrozooplankton meals as fish meal replacers (**Paper II**). Inclusion of krill (>20% of diet proteins) and replacing Carophyll Pink® with natural astaxanthin esters from the krill meal resulted in higher muscle whiteness and lower colour hue and -intensity in cooked salmon cutlets scored by panellists compared to salmon fed control feed. Technical colour assessment measured by colorimeter-HunterLab pointed in same direction by giving lowest redness (a*) and chroma values for salmon that was fed with high inclusion levels of krill meal and the lowest level of synthetic Carophyll Pink®. However, fish groups replacing 20% fish meal with northern krill proteins or 40% with amphipod

proteins resulted in higher a*- and chroma values compared to control fish meal group, probably reflecting highest feed intake and hence astaxanthin in these groups. The fish from these groups were slightly heavier compared to other experimental groups.

Sensory taste analysis resulted in a lower salty-taste score for fish fed Antarctic krill than the amphipod and control groups, whereas the amphipod group scored lowest for bitter taste, significantly different from Antarctic krill meal group and northern krill group (40% of dietary proteins, **Paper II**). Differences between experimental groups receiving krill or amphipods were however marginal, suggesting that fish meal can successfully be replaced with macrozooplankton for up to 60% without affecting any negative sensory experiences of the fed salmon.

Wild Atlantic cod (2.3-6.7 kg) deviated from farmed cod (mean of 4.8 kg) in 13 of 22 investigated sensory attributes. Farmed cod had a tendency to have less intense taste scores, whereas taste parameters including intensity, bitterness, stale-, metal-, seaweed- and watery taste did not differ significantly between wild or any farmed fish groups fed different levels of Antarctic krill meal (0-100%) (**Paper IV**). Additionally, sensory texture of wild cod showed more flakiness and shininess, and was softer and juicier than the farmed cod groups. Wild cod was judged to have a higher acidulous taste, more sea taste and less saltiness and seabed taste than farmed cod, independently of Antarctic krill inclusion level in their diets (**Paper IV**). There were no differences in sensory quality attributes in cod fed the control fish meal diet and fish fed diets where fish meal was replaced with Antarctic krill meal (0-100%). Based on this study it can be concluded that the inclusion of Antarctic krill meal in cod diets does not move the sensory attributes more towards those of wild cod. Differences observed between wild and farmed cod may therefore stem from different feeding regime, growth rate and diet composition (moisture content in natural feed vs. pellets) rather than differences in protein quality between these two groups. The diet and energetic status of the wild cod were not known.

The muscle colour of farmed cod showed higher L* values than the wild cod group, and this may reflect the higher dry matter content and concomitant reduced transparency (Mørkøre, 2005; Stien *et al.,* 2005) of farmed cod. Also lower *post mortem* pH in farmed cod is linked to increased muscle whiteness (Mørkøre, 2005). Soya oil in Atlantic cod diet has been reported to result in negative darker muscle colour (Mørkøre, 2005). Light muscle colour is considered as a good quality parameter in cod, and was seen in all farmed cod in **Paper IV** independent of protein source fed.

Wild cod skin colour is reported to be lighter than farmed ones (Mørkøre, 2005) and there was a tendency for higher lightness (L*) values for wild cod than for farmed ones when measured with a colorimeter in **Paper IV**. Atlantic cod is known to change its skin colour after environment and diet. The skin of wild cod was significantly more yellow (b*) than farmed cod. Inclusion of macrozooplankton in the diets of farmed cod indicated a redder (a*) hue in the skin compared to the control fish meal diet without astaxanthin supplementation.

4.6. Fish health and consumer's safety

In addition to good growth and end quality of farmed fish, fish health and welfare are essential in aquaculture. Feeding with sub-optimal diets affects fish physiology, and may lower the nutritional status leading to growth reduction, sickness and mortality (Waagbø, 2006). Optimal diets do not only provide all essential nutrients, but nutrients may also act as immunomodulators. Good growth and lack of mortalities in the feeding experiments indicated that fish welfare was not compromised (**Papers I-V**). Additionally, haematological tests and clinical analysis like the plasma enzymes aspartate amino-transferase (ASAT) and alanine amino-transferase (ALAT) values were all within reported normal ranges and confirmed a good health of the fish in all experiments (Sandnes *et al.,* 1988). This is in accordance with an earlier report by

Haig-Brown (1994) where krill meal based diets were demonstrated to even increase disease resistance in farmed salmon. Inclusion of krill meal in the diet has also been suggested to inhibit fin erosion, probably due to its high mineral concentration (Logva, 1989; Lellis & Barrows, 1997).

The exoskeleton of crustaceans constitute a high level of fluoride, up to 1000-6000 mg kg^{-1} dry matter (Soevik & Brækkan, 1979; Sands *et al.,* 1998) and other undesirable elements (Ritterhoff & Zauke, 1997; 1998) that may restrict their direct use for human consumption and in fish diets, given upper limits in the current EU legislation (EC Directive 2003). This legislation also covers raw materials from land based production systems where the content of these minerals is naturally low. One of the reasons for the declining krill fisheries at the beginning of 1980s was the high fluoride content in krill. This problem was, however, partly solved when it was shown that fluoride was not deposited in edible parts in fish (Grave, 1981; Tiews *et al.,* 1982), but rather in scales and bones. The EU has set a maximum limit for fluoride content in animal feed, including feed for fish, at 150 mg kg^{-1} (EC Directive 2003). A recent study showed that Atlantic salmon did not deposit fluoride when the dietary fluoride content ranged from 18 to 358 mg kg^{-1} (Julshamn *et al.,* 2004). The earlier studies showing fluoride accumulation in fish bones may therefore have been exceptions rather than the normal case. Fish that ingest crustaceans for part of their lives may be adapted to a similar exposure of elements (Jacobsen & Hansen, 1996); this was confirmed in the study with Atlantic salmon where biological availability of fluoride was low (Julshamn *et al.,* 2004). However, fish reared in freshwater might respond differently as freshwater fish accumulate minerals in the body whereas fish in seawater have to excrete them to adjust for osmotic differences in body fluids and seawater (Yoshitomi *et al.,* 2006). That was seen in accumulation of fluoride in the bones resulting in skeletal deformities, significantly lower weight gain, feed intake and SGR, when Antarctic krill meal replaced for up to 30% of fish meal of small rainbow trout reared in fresh water (Yoshitomi *et al.,* 2006). Lower survival rates and a higher level of skeletal deformities were also seen by Opstad *et al.* (2006), when fish meal substitution with amphipod meal exceeded 50% in juvenile cod diet.

Deformities may have occurred due to the high element and ash concentrations found in amphipod meal and the subsequent lower available protein content and insufficient essential amino acid content for juvenile fish. Roem & Kelly (1991) reported lower growth in post-larval chum salmon (*O. kisutch*) (0.41 g) when fish meal was fully replaced with Antarctic krill meal. Lower growth was explained due to vitamin deficiencies affected by probably manufacturing practises in krill meal in that study. No deformities were seen in the feeding studies in this thesis when using Atlantic salmon, -halibut or -cod with bigger fish size in seawater.

Elements may act both as essential nutrients and as toxicants in fish feeds depending on the metal, chemical form and level. Potentially toxic elements occur naturally in the environment and are present in feed ingredients, or originate from pre-mix supplementation in complete feeds. A detailed discussion and an account of the maximum tolerate concentrations of essential metals in fish feeds are presented in *Mineral Tolerance of Domestic animals* (NRC, 1980). **Paper V** focuses the non-essential undesirable element level in meals and complete diets prepared from northern krill, Antarctic krill and amphipods. For future use of zooplankton meal, it is essential to document the levels of undesirable substances in the meals and fish diets prepared from them, and possible risks for consumers of the produced seafood.

The levels of zinc (Zn), arsenic (As) and lead (Pb) in meals prepared from krill and amphipod were within the same range as reported earlier by Li *et al.* (2005), Edmonds (2003), Sydeman & Jarman (1998) and Ritterhoff & Zauke (1997). These elements were, together with mercury (Hg), at similar or lower levels than those found in fish meal and far below the EU's respective upper limits (Figure 7). Arsenic levels are often high in fish meals (3.6–18.2 mg kg^{-1} dm; Sloth *et al.,* 2005) where the EU's upper limit for feed ingredients is 15 mg kg^{-1} (88% dm). The As level in Antarctic krill meal was 4 mg kg^{-1} (dm), 9.4 mg kg^{-1} (dm) in northern krill and 11 mg kg^{-1} (dm) in amphipod meal (Figure 7; **Paper V**). Using meals prepared from macrozooplankton as fish meal replacement does not exceed any restrictions regarding these element concentrations. The chemical composition of zooplankton

including its metal concentration has been shown to have a large variation depending on species, catch area, season and grazing period. Similarly, fish species, season, diet, life-stage and catching area have a major impact on the element concentration in meals prepared from them (EFSA, 2005). The Hg levels is reported to range between 0.12–0.23 mg kg^{-1} dm in macrozooplankton species in Northern Waters (Ritterhoff & Zauke, 1997), which is much higher than our findings in **Paper V** where it ranged between 0.008-0.04 mg kg^{-1} (Figure 7). The EU's upper limit for Hg is 0.5 mg kg^{-1} (88% dm).

The level of copper (Cu) is naturally high in krill since Cu is found in the blood haemocyanin of crustaceans, functioning as an oxygen carrier equivalent to haemoglobin in fish (Bridges *et al.,* 1983). Cu levels ranged between 8 and 46 mg kg^{-1} dm (Figure 7; **Paper V**) being highest in meal prepared from *E. superba,* and exceeded the EU upper limit of 25 mg kg^{-1} (88% dm). These Cu levels were within the range found earlier by Ritterhoff & Zauke (1997) and Sydeman & Jarman (1998). However, they found a higher Cu content in amphipod from the Greenland Sea than was found in our study in amphipod caught from Jan Mayen. This variation may again be due to the different harvesting area. The level of cadmium (Cd) was highest in amphipod meal (12 mg kg^{-1} dm) (Figure 7), and may relate to the detoxification system with metal-binding proteins in amphipods as an adaptation to the high dietary element levels, as suggested by Ritterhoff & Zauke (1998). The level of Cd was 6 times higher than the EU's upper limit for feed ingredients (2 mg kg^{-1} 88% dm) and may restrict the use of this meal in fish diets within the current legislation.

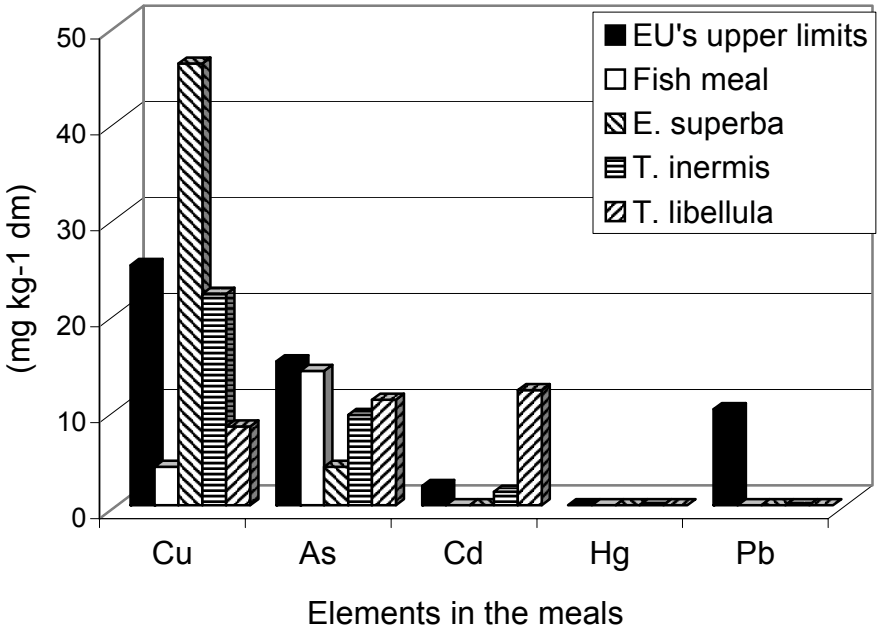


Figure 7. *Dry matter (dm) concentration of undesirable elements in meals prepared from fish (LT-meal) and macrozooplankton, and the EU's upper limits for feed ingredients. Level of zinc (132-147 mg kg $^{-1}$) was below the maximum limit of 200 mg kg $^{-1}$.*

Diet element levels are shown in Figure 8 and reflected the levels found in meals. High total arsenic (As) level was found in fish meal and exceeded the EU maximum content of 6 mg kg^{-1} (2 mg kg^{-1} for inorganic arsenic) in complete feed. As levels were lower in macrozooplankton meals, and showed generally lower values in complete diets including macrozooplankton meals (Figure 8). Lowest level of As was found in the diet where fish meal was fully replaced with *E. superba* meal. On the other hand, including Antarctic krill meal in the diet at levels 40-100% of diet proteins, the EU maximum limit for Cu (25 mg kg^{-1}) was exceeded (27-52 mg kg^{-1}) in the complete diets. Similarly, high Cd levels in amphipod meal caused excessively high Cd concentrations in the complete diet with 40% replacement of diet protein (Figure 8). The dietary level of Hg and Pb were low and both under detection limit (**Paper V**) and EU upper limits.

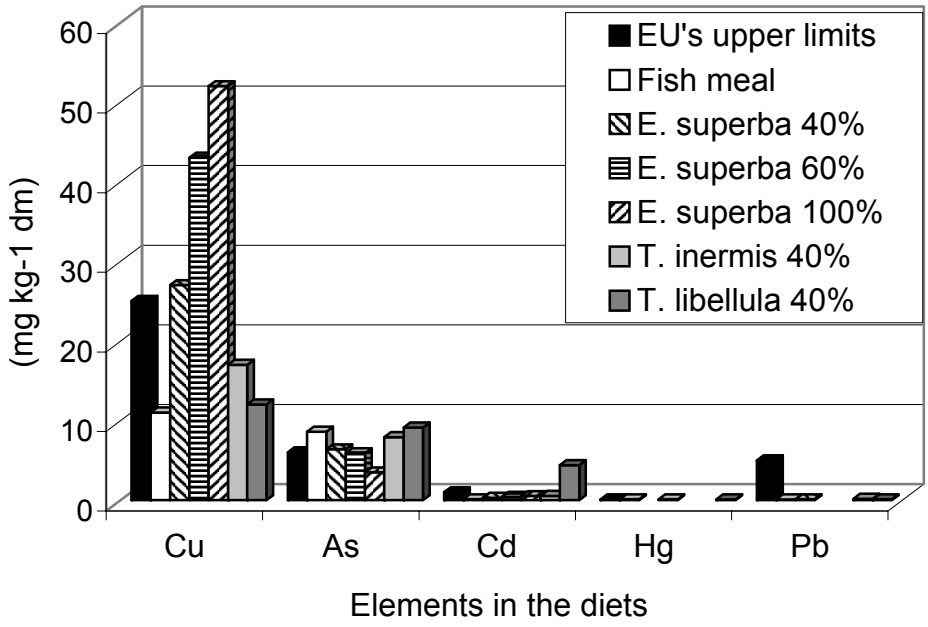


Figure 8. *Dry matter (dm) concentration of undesirable elements in diets prepared from fish (LT-meal) and macrozooplankton (% of diet proteins), and the EU's upper limits for complete feed (88% mg kg^{-1} diet dm). Level of zinc (132-200 mg kg^{-1}) was below the maximum limit of 200 mg kg^{-1}.*

Feeding with a high dietary Cu level (34 mg kg^{-1} dm) has shown negative effects for fish health (Berntssen *et al.,* 2000). An other study showed Atlantic salmon to be highly tolerable for high cadmium exposure 250 mg kg^{-1} for 4 months without adverse effects on morphology or growth (Berntssen *et al.,* 2001). Highest dietary levels found in the amphipod diet was 4.4 mg kg^{-1} diet (dm) (**Paper V**). The maximum permitted limit for Cd in the EU has been set as 1 mg kg^{-1} diet (dm) in animal feed (EC directive 2005/87/EC).

The muscle of Atlantic salmon and cod fed with macrozooplankton based diets in terms of Zn, Hg and Pb concentrations remained unaffected after 160 and 75 days feeding, respectively (Figure 9; **Paper V**). Elements like Cu and Cd are reported to accumulate in the viscera (intestine, liver and kidney) rather than in fish muscle (Berntssen *et al.,* 1999; 2000; 2001). Likewise, Pb is reported to accumulate in

internal organs, skin and bones rather than fish muscle tissue (Somero *et al.,* 1997). No significant accumulation of Cd in salmon fish muscle was reported at dietary concentrations up to 5 mg kg^{-1} dm (Berntssen *et al.,* 2001). The content of Cd and Pb were under the detection limit in the muscle (**Paper V**). The Cd and Pb content in farmed salmon fillets have been shown to be under 0.001 and 0.01 mg kg^{-1}, respectively (Maage *et al.,* 2005). The EU maximum contents for these elements in food for human consumption are 0.05 and 0.2 mg kg^{-1} muscle dm, respectively (EC Directive 2003). Muscle content of Hg in the present study ranged between 0.02-0.03 mg kg^{-1} muscle dm, and was below the EU maximum limit of 0.05 mg kg^{-1} muscle dm, independently of zooplankton meal source used (**Paper V**). These values are in agreement with Maage *et al.* (2005) who reported that mean mercury content ranged between 0.015 and 0.039 mg kg^{-1} (ww) in farmed Atlantic salmon.

The only effect seen in the muscle element level was the lower arsenic (As) level (0.4-1.0 mg kg^{-1} muscle dm) in fish fed the macrozooplankton based diets compared to fish fed the control fish meal diets (4.1-4.5 mg kg^{-1} muscle dm). A lower As level in seafood is desirable, as the levels are often high in seafood. A level of As in farmed salmon fillet has been monitored regularly in Norway since 1995 and the levels have declined from the mean of 3.3 mg kg^{-1} (ww) to 1.6 mg kg^{-1} (ww) in 2001 (Julshamn *et al.,* 2003). Marine organisms accumulate arsenic predominantly as non-toxic arsenobetaine and arsenocholine that is not considered to represent a significant health risk (Sloth *et al.,* 2005). There are no reports of toxicity in man or animals from consumption of organoarsenicals in seafood (EFSA, 2005). The National Institute of Nutrition and Seafood Research (NIFES) has recommended increasing the maximum level of arsenic in fish feed to 10 mg kg^{-1}.

There are no current EU maximum levels for Cu or Zn in food for human consumption. Based on this study, it can be concluded that macrozooplankton can successfully be included in the diets of Atlantic salmon and cod without increasing the Cu, Zn, As, Cd, Hg or Pb levels in the muscle, and therefore including macrozooplankton in fish feed does not possess any risk for consumers.

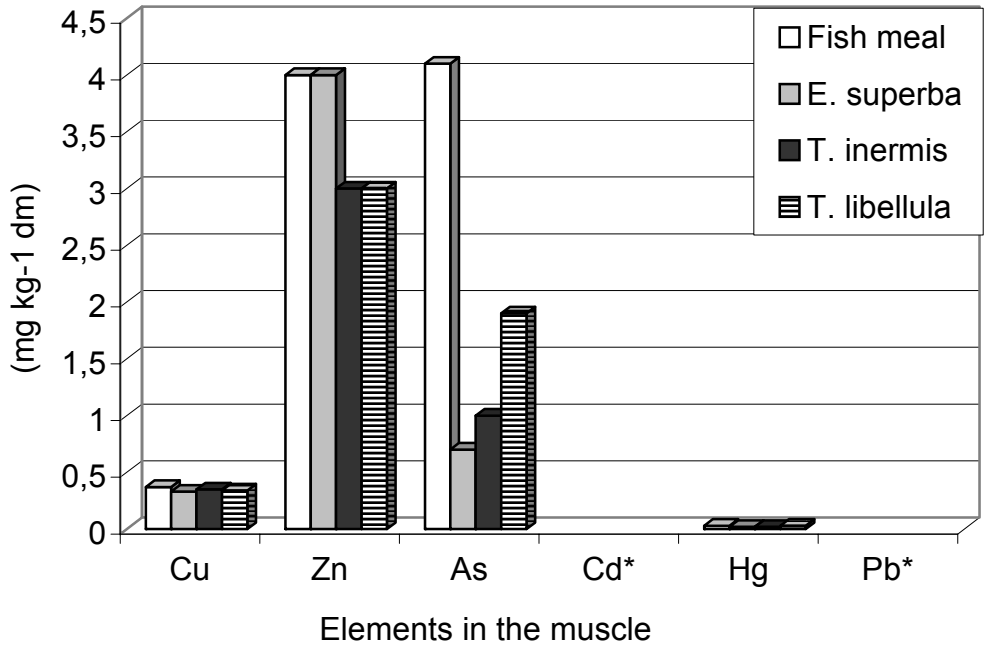


Figure 9. *Dry matter (dm) concentration of undesirable elements in Atlantic salmon muscle fed with fish meal (LT-meal) and zooplankton for 160 days. The level of Cd and Pb were below the detection limits of the method (*).*

In order to be able to guarantee the safety of seafood, national food authorities (the Norwegian Food Safety Authority) offer dietary advice on the consumption of seafood. The risk assessments, apart from those for mutagenic substances, are based on tolerable weekly intake (TWI) levels (TWI values are not given for mutagenic substances, *i.e.* substances that may produce changes in our genetic material, since any ingestion of such substances increases the level of risk).

The TWI values employed in connection with evaluations of diet advisories are as follows and based on the Joint FAO/WHO Expert Committee on Food Additives (JECFA):

- Inorganic arsenic (As) - 15 μg kg^{-1} body weight per week
- Lead (Pb) - 25 μg kg^{-1} body weight per week

- Cadmium (Cd) - 7 μg kg^{-1} body weight per week
- Methyl mercury (Hg) - 1.6 μg kg^{-1} body weight per week

The levels of these undesirables in Atlantic salmon muscle were low as seen in Figure 9 (**Paper V**). Arsenic is present mostly as non-toxic form, and cadmium and lead were under detection limit (**Paper V**), and these elements are generally considered insignificant risk contributor for human health via finfish (EFSA, 2005). Methyl mercury does not accumulate in fatty fish, but concentrates in fish with increasing age being low in fast growing aquaculture species. Fish is important source of high quality protein, ω-3 PUFA, and certain vitamin and minerals, and one to two portions of seafood (high in ω-3 PUFA) are recommended for potential benefits to human health.

Organic pollutants (PCBs, dioxins, organochlorines) accumulate in the lipid reserves of organisms from lower trophic levels towards higher trophic position (Borgå *et al.,* 2001; 2005) and the level of these compounds are lower in krill compared to fish (Landrum & Fisher, 1998; Sclabos & Toro, 2003). Organic contaminants were not included in this study, but need further investigations. As there has been an increased public concern about these pollutants, this provides additionally support for the suitability of feed raw materials from lower trophic levels for use in aquaculture feeds.

5. Conclusions

5.1. Raw material and diet properties

Macrozooplankton meals had a lower protein content, a higher chitin and ash content and similar or higher lipid content compared to fish meal. Hence, higher inclusion levels than fish meal had to be added in complete diets to reach comparable protein level. Feed fatty acid and essential amino acid compositions remained unaffected independently of meal source used and all fulfilled the established requirements for cold water fish. Inclusion of macrozooplankton, especially krill, contributed significantly to the dietary total astaxanthin content. The diet with the highest levels of macrozooplankton gave slightly softer feed pellets meaning that the diet processing technology has to be developed further to optimalise the feed properties with high dietary zooplankton levels.

5.2. Feed conversion, growth and nutrient digestibility

A higher FCR was seen in Atlantic salmon when the Antarctic krill inclusion level increased to >80% of diet proteins. Inclusion of krill up to 60% or amphipod up to 40% of diet proteins did not affect FCR significantly. Amphipod and northern krill significantly increased the SGR during the whole experimental period in adult salmon and halibut, respectively. Apparent digestibility of lipid was slightly lower in salmon when >80% of fish meal proteins was replaced with Antarctic krill meal. Protein and dry matter AD remained unaffected independently of protein source used. Chitin digestibility was low in Atlantic salmon, but showed a slight increase with increasing dietary chitin level.

5.3. Muscle quality

The marine fatty acid profile with high EPA and DHA was maintained or even improved in Atlantic salmon muscle when fish meal was replaced with macrozooplankton. Sensory taste, odour, texture, and fillet astaxanthin content remained unaffected, but salmon colour was slightly negatively affected when fish meal was replaced with macrozooplankton. Wild cod deviated from farmed cod, but inclusion of Antarctic krill meal did not deviate from cod fed a fish meal diet with respect to muscle pH, texture or sensory attributes.

5.4. Undesirables, seafood safety and fish health

The elements Zn, As, Pb and Hg in macrozooplankton meals and in complete diets were lower than the current EU's upper limits, but were exceeded for Cu and Cd in Antarctic krill and amphipod meal, respectively, in meals and complete diets. However, no element accumulated in either Atlantic salmon or cod muscle. Up to 40% fish meal replacement by *T. inermis*, *T. libellula* or *E. superba* meals in feeds for Atlantic salmon, and up to 100% *E. superba* meal in Atlantic cod diet did not possess any risk for consumers, as evaluated from muscle element concentrations. The experimental fish showed good growth, normal blood and plasma values of health related parameters, and absence of mortality in all feeding experiments.

6. Future perspectives

Currently, the utilisation of macrozooplankton in the Norwegian and Barents Seas is low or negligible. Only small-scale local copepod (*Calanus*) fisheries have been reported in costal Norway; it is used for feed raw materials and as a pigment source for salmonids. The high macrozooplankton meal price (present price about the twice of fish meal) has been the most limiting factor for using them as fish meal and fish oil replacement. Antarctic krill fisheries have, however, shown great promise for expansion as the marine resources for fish feeds are scarce (Nicol & Foster, 2003). New technologies for macrozooplankton fisheries have been developed (SC-CCAMLR 2004), and have enabled increased catch volumes, on-board processing and higher product value. A rapid expansion of krill fisheries in the Southern Ocean has raised concerns about the future of still less well understood marine ecosystems (SC-CCAMLR 2005b). Therefore, harvesting zooplankton should always be based on current scientific based recommended levels.

There are still challenges with the use of macrozooplankton in fish feeds. The carapace of zooplankton contains high fluoride content, krill (particularly Antarctic krill) contain a high copper level and Arctic amphipod contains a high cadmium level. These substances may set limits as to what extent meals prepared from them can replace fish meal within the current EU legislation. Additionally, the carapace results in high level of indigestible matter (chitin, ash) in meals lowering the dietary energy content, physical properties, and may restrict the use of meals at higher inclusion levels (>80% of proteins) as shown in **Paper I**. However, the influence of chitin in diets for farmed fish is still unclear and needs more research. More studies of these mechanisms for digestibility, growth and health of fish are needed and are presently under way. Raw material handling by peeling raw zooplankton may be one solution to reduce unwanted substances from crustaceans. On the other hand, **Paper V** showed, that elements that were found in macrozooplankton did not accumulated in fish muscle, and therefore do not possess any risk for consumers or fish health. More studies are needed to document the suitability of different zooplankton species

with different dietary levels. Maximum allowed elements content in complete feedingstuffs should be based on the chemical species of toxicological relevance. Increased research and knowledge on element species may contribute to more realistic upper limits for marine specific undesirables.

Besides, the processing of raw material is challenging as raw zooplankton deteriorates within the first hours after being caught. Ensilage, fermenting and lipid extractions may offer some interesting research areas for effective and cheap handling and processing of raw materials to keep the high quality of the zooplankton.

When evaluating the use of zooplankton for feed production there is the additional challenge of fully characterising the chemical composition of the different zooplankton species, as the composition varies depending on availability of food, sex and age of the animal, geographical region and the time of the year. Also, the optimal krill meal level that is sufficient for salmon pigmentation should be evaluated. In **Paper II**, the highest krill inclusion level in salmon diets was 60%. Since this level did not give optimal pigmentation evaluated by sensory panel, it would be interesting to study how full fish meal replacement by krill meal will affect pigment digestibility and muscle pigmentation in salmonids.

Furthermore, more studies are needed to evaluate the optimal inclusion level in diets using different zooplankton species as feed source for fish species in different life-stages. Involving self-feeding devices to study taste preferences and feed intake in fish would give much interesting research areas as well. Diets should be tested in large scale feeding experiments under commercial conditions. From the discussed properties of zooplankton meals, combinations with less optimal raw materials of vegetarian origin (protein and lipid sources) give interesting perspectives and possibilities as alternatives for fish meal and fish oil.

In summary, macrozooplankton represents an enormous potential for use in aquaculture feed, as these resources are mainly underutilised, and feeding studies have shown the same or better growth performance when high quality fish meal was replaced with macrozooplankton meals. Because of the current higher price and higher element concentration found in macrozooplankton meal compared to fish meal, it is recommended that macrozooplankton meal be primarily used as feed attractants, sources of natural carotenoids and ω-3 fatty acids rather than main or sole protein source in fish feeds. Harvesting from lower trophic levels decreases the pressure on over-exploited fishing resources, as currently the carnivorous aquaculture industry relies on restricted fish resources as the main source of protein and oil. However, to find economically profitable and dense swarms to harvest significant amounts of zooplankton, the Antarctic Ocean where the biomass and area is huge, is the most probable alternative for fisheries, rather than in the Norwegian and the Barents Sea. Although there is increase knowledge of distribution and abundance of zooplankton, there is still limited information about the relationship between zooplankton and other species, and how environmental conditions, such as climate change, affect zooplankton populations and zooplankton dependent species. Hence, zooplankton fisheries management has to base on science, where ecosystem is seen as a whole and where sustainable harvesting can be maintained.

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