European Organic Aquaculture - Science-based recommendations for further development of the EU regulatory framework and to underpin future growth in the sector

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Chapter 1: FEED REQUIREMENTS

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Authors:
Alfred Jokumsen (DTU)
Giuseppe Lembo (COISPA)
Gerd Marit Berge (Nofima)
Grete Bæverfjord (Nofima)
Zdeněk Adámek (USB)
Cyrille Przybyla (Ifremer)
Wout Abbink (IMARES)

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Authors:
Chris Noble (Nofima)
Bjørn-Steinar Sæther (Nofima)
Giuseppe Lembo (COISPA)
Zdeněk Adámek (USB)
Alfred Jokumsen (DTU)
Åsa Maria O. Espmark (Nofima)
Hans van de Vis (IMARES)
Grete Bæverfjord (Nofima)
Walter Zupa (COISPA)
Pierluigi Carbonara (COISPA)
Maria Teresa Spedicato (COISPA)
Eleonora Fiocchi (ISZVe)
Amedeo Manfrin (ISZVe)
Jean-Paul Blancheton (Ifremer)
Emmanuelle Roque d'Orbcastel (Ifremer)
Helena Röcklinsberg (SLU)
Ainhoa Blanco (IMARES)
Wout Abbink (IMARES)

Chapter 3: PRODUCTION SYSTEMS

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Authors:
Giuseppe Lembo (COISPA)
Zdeněk Adámek (USB)
Alfred Jokumsen (DTU)
Chris Noble (Nofima)
Bjørn-Steinar Sæther (Nofima)

Hanne Marie Nielsen (Nofima)
Ingrid Olesen (Nofima)
Helena Röcklinsberg (SLU)
Myriam Callier (Ifremer)
Jean-Paul Blancheton (Ifremer)
Emmanuelle Roque d'Orbcastel (Ifremer)
Wout Abbink (IMARES)

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Authors:
Zdeněk Adámek (USB)
Alfred Jokumsen (DTU)
Chris Noble (Nofima)
Bjørn-Steinar Sæther (Nofima)
Turid Synnøve Aas (Nofima)
Trine Ytrestøyl (Nofima)
Helena Röcklinsberg (SLU)
Ingrid Olesen (Nofima)
Wout Abbink (IMARES)

Project management Board of OrAqua:

Ingrid Olesen (Nofima)
Åsa Maria O. Espmark (Nofima)
Giuseppe Lembo (COISPA)
Jean Paul Blancheton (Ifremer)
Pirjo Honkanen (Nofima)
Wout Abbink (DLO-Imares)
Alfred Jokumsen (DTU)
Magnus Ljung (SLU)
Sriskandarajah Nadarajah (SLU)

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1. Introduction and current regulations

(EC 834/2007, rec. (1)): In perspective of the regulatory framework organic production is a *”system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes. The organic production method thus plays a dual societal role, where it on the one hand provides for a specific market responding to a consumer demand for organic products, and on the other hand delivers public goods contributing to the protection of the environment and animal welfare, as well as to rural development.”*

EC 834/2007, rec. (9); Regarding feed and nutritional issues the current regulation states that *”genetically modified organisms (GMOs) and products produced from or by GMOs are incompatible with the concept of organic production and consumers' perception of organic products. They should therefore not be used in organic farming or in the processing of organic products.”*

EC 834/2007, rec. (15d: *”Feed for fish and crustaceans shall meet the animal's nutritional requirements at the various stages of its development. The plant fraction of the feed shall originate from organic production and the feed fraction derived from aquatic animals shall originate from sustainable exploitation of fisheries. Non-organic feed materials from plant origin, feed materials from animal and mineral origin, feed additives, certain products used in animal nutrition and processing aids shall be used only if they have been authorised for use in organic production under EC 834/2007, Art. 16. Growth promoters and synthetic amino-acids shall not be used”.*

EC 834/2007, rec. (16 2(e) (ii): *”Feed of mineral origin, trace elements, vitamins or provitamins shall be of natural origin. In case these substances are unavailable, chemically well-defined analogic substances may be authorised for use in organic production.”*

According to Commission Regulation (EC) No 889/2008, art. 25j, *feeding regimes shall be designed with the following priorities: (a) animal health, (b) high product quality, including the nutritional composition which shall ensure high quality of the final edible product; (c) low environmental impact.*

EC 889/2008, art. 25k: *“Feed for carnivorous aquaculture animals shall be sourced with the following priorities: (a) organic feed products of aquaculture origin; (b) fish meal and fish oil from organic aquaculture trimmings; (c) fish meal and fish oil and ingredients of fish origin derived from trimmings of fish already caught for human consumption in sustainable fisheries; (d) organic feed materials of plant or animal origin.”*

As regards shrimps, the Reg. 889/2008, art. 25l, par. 3, says that “*where natural feed is supplemented according to paragraph 2 the feed ration of species as mentioned in section 7* *of the Annex XIIIa (penaeid shrimps) may comprise a maximum of 10% fish meal or fish oil derived from sustainable fisheries".*

2. State of the art fish meal, fish oil, and mineral and vitamin supply

Organic aquaculture is a specific production approach (Cottee and Petersan, 2009) driven by the growing interest in sustainable utilization of resources (Mente et al., 2011). There is increasing concern about the consumption of fish meal and fish oil for aquaculture feed due to the increasing demand from the expanding aquaculture industry and concerns about decreasing wild stocks. The current European regulation on organic aquaculture (Commission Regulation (EC) No 889/2008) does not allow fish meal and fish oil derived from traditional industrial fish, but only from trimmings of fish from organic aquaculture or from trimmings of fish already caught for human consumption in sustainable fisheries, in order to prevent reductions in fish stocks. However, Commission Regulation (EC) No 834/2007, Art. 15 Production rules for aquaculture animals, "(d) with regard to feed for fish and crustaceans states that "Animals shall be fed with feed that meets the animal's nutritional requirement at the various stages of its development". Still, the organic regulation does not allow balancing the dietary amino acid profile by supplementing with synthetic free amino acids to fulfil the dietary requirements of the specific organically produced species.

In fresh water ponds, the detritus, colonies of bacteria, aquatic weeds, plankton and terrestrial and water insects and their aquatic larvae are all natural food for the different fish species. In pond polyculture, the role of natural fish food is outstanding because it is the source of protein in the diet of fish which otherwise would only be supplied by expensive fish meal.

2.1. Fish meal replacement

It is a fact that fish meal of high quality provides a balanced amount of all essential amino acids, minerals, phospholipids and fatty acids reflected in the normal diet of fish (Hardy, 2010; Lund et al., 2012), and hence secure high utilization by the fish and minimum discharge of nutrients to the environment.

In particular, a diet based on marine sources secures optimum development, growth and reproduction, especially of farmed larvae and brood-stock. Fish oil is a major natural source of the long chain omega-3 HUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can be synthesized by salmonids and by other carnivorous marine species only at a limited rate, and thus are required in the diet. Omega-3 HUFAs are produced by marine phyto- and zooplankton, which are consumed by the wild marine fish larvae (Baron et al., 2013). Hence, fish meal and fish oil are strategic ingredients to be used at critical stages of the life-cycle when optimum performance is required.

2.1.1. Curent regulations

According to Commission Regulation (EC) No. 889/2008; Art. 25k, the feed ingredients (mainly fish meal and – oil) shall be sourced in priority:

"1. (a) organic feed products of aquaculture origin; (b) fish meal and fish oil from organic aquaculture trimmings; (c) fish meal and fish oil and ingredients of fish origin derived from trimmings of fish already caught for human consumption in sustainable fisheries; (d) organic feed materials of plant or animal origin."

2. If feed mentioned above (1a – 1d) is not available, fish meal and fish oil from non-organic aquaculture trimmings, or trimmings of fish caught for human consumption may be used for a transitional period until 31 December 2014. Such feed material shall not exceed 30% of the daily ration.

3. The feed ration may comprise a maximum of 60% organic plant products.

4. Astaxanthin derived primarily from organic sources, such as organic crustacean shells may be used in the feed ration for salmon and trout within the limit of their physiological needs. If organic sources are not available natural sources of astaxanthin (such as Phaffia yeast) may be used.

According to Commission Regulation (EC) No. 889/2008; Art. 25l, par. 3 *"Where natural feed is supplemented according to paragraph 2 the feed ration of species as mentioned in section 7 of the Annex XIIIa (penaeid shrimps) may comprise a maximum of 10% fish meal or fish oil derived from sustainable fisheries".*

2.1.2. Current scientific knowledge

Replacing fish meal in diets for salmonids and marine species is not straightforward due to their unique contents of protein, excellent amino acid profile, high nutrient digestibility, high palatability, adequate amounts of micronutrients, as well as general lack of anti-nutrients in fish meal (Gatlin et al., 2007; Kaushik and Seiliez, 2010; Krogdahl et al., 2010; Lund et al., 2012). Moreover, compared to salmonids, protein requirements of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) are higher, reflecting their highly carnivorous nature (Oliva-Teles, 2000).

A large number of studies have investigated the effects of replacing fish meal with various plant protein ingredients (Altan and Korkut, 2011; Borquez et al., 2011; Glencross et al., 2011; Lanari and D'Agaro, 2005; Pereira and Oliva-Teles, 2002; Pratoomyot et al., 2010; Sitjà-Bobadilla et al., 2005; Torstensen et al., 2008; Yang et al., 2011). Complete replacement by plant proteins is usually not successful due to problems related to the anti-nutrient factors, altered patterns of amino acid uptake when replacing fish meal with plant based protein ingredients, and impairment of immune competence (Bendiksen et al., 2011; Borquez et al., 2011; Espe et al., 2006; Francis et al., 2001; Gatlin et al., 2007; Geay et al., 2011; Lanari and D'Agaro, 2005; Larsen et al., 2012; Lund et al., 2011; Sitjà-Bobadilla et al., 2005). Concerning marine carnivorous species, studies on gilthead sea bream showed that 75% of protein could be provided by a large vegetable sources (corn gluten, wheat gluten extruded peace and rapeseed) without compromising digestive process, but the fish feed uptake significantly decreases up to this percentage (Santigosa et al., 2011b). A total substitution induced a strong reduction of the protease activity.

High replacement ratios require that anti-nutrients (such as trypsin inhibitors, tannins, lectines or glucosinolates) (Chebbaki et al., 2010) are efficiently removed from alternative plant protein ingredients to meet the high protein requirement of fish. The dietary content of indigestible substances should be minimized to optimize the efficiency of the feed.

Extrusion processing could be used to obtain vegetable products with extremely low levels of heat sensitive anti-nutritional factors and to increase the nutritional value of protein-containing ingredients (Chebbaki et al., 2010). Not all anti-nutrients are destroyed by heat treatment. Gossypol from cottonseed, glucosinolates in rapeseed meal, and phytic acid in soybean, rapeseed and cottonseed meals are examples of harmful substances that needs to be controlled by other means (Hardy and Barrows, 2002). Furthermore, it is necessary to ensure that the dietary amino acid profile is optimised, for example by adding free amino acids, and/or by combining several plant protein sources with different amino acid composition (Francis et al., 2001; Kaushik and Seiliez, 2010; Wilson, 2002). The use of agar coated crystalline-amino acids in sea bream juveniles proved to improve the leaching and the delay absorption from the digestive tract (Peres and Oliva-Teles, 2009). Recent studies at BioMar have shown that it is possible to include as little as 5% fish meal in addition to various vegetable protein concentrates supplemented with free amino acids for feed for salmonids without negative effects on performance (Ekmann, 2014).

Nutrient requirements were reported in NRC (2011) “Nutrient Requirements of Fish and Shrimp”. However, most of the data were obtained with juvenile and larval fish, under conditions regarded optimal. There were no surpluses in the requirement data reported. A further safety margin is needed for nutrient loss in feed production, variation in content in feed ingredients, interactions between nutrients or ingredients, and increased requirements in certain situations (environmental stressors, infections etc.). Requirements may also vary in different life stages of the fish.
The requirements for amino acids, fatty acids, vitamins and minerals were determined with diets containing purified and chemically defined ingredients highly available to the fish. Nutrient bioavailability is variable in different feed ingredients, and needs to be evaluated for every feed ingredient.

Fish do not have absolute protein requirements, but require the amino acids that compose the proteins. Atlantic salmon (*Salmo salar*) has documented requirements for the amino acids Arg, His, Ile, Leu, Lys, Met, Cys, Phe, Tyr, Thr, Try and Val, while Tau is not regarded as required (NRC 2011). Recent publications indicate higher requirements of lysine and threonine at the smolt stage (Grisdale-Helland et al., 2011; 2013), than the requirements reported as mean values by NRC. This may also be the case for other amino acids.
The essential amino acid (EAA) requirements for optimal growth of Mediterranean fish species, such as sea bass, are Lys, Arg, Met, Cys, Try and Thr (Tibaldi and Kaushik, 2005). Peres and Oliva-Teles (2009) reported that the optimal balance of essential amino acids in the diets for gilthead sea bream juveniles expressed relative to lysine (=100) A/E ratios (where A0 the specific essential AA and E the total of essential AA) were estimated to be: arginine, 108.3; threonine, 58.1; histidine, 36.8; isoleucine, 49.7; leucine, 92.7; methionine, 50.8; phenylalanine+tyrosine, 112.3; valine, 62.6; and tryptophan, 14.6. This EAA profile correlates tightly to the whole-body EAA composition of gilthead sea bream.

Dietary amino acid disproportions may be regarded as the primary cause of changes in feed consumption and there is some evidence that voluntary feed intake in sea bass may be partially conditioned by limiting or excessive levels of certain diet EAA (Tibaldi and Kaushik, 2005). Diets with lower proportions of tryptophan resulted in loss of appetite to juvenile seabass, while diets lacking in methionine induced a reduction in feed intake (Thebault et al.,1985). Diets lacking in tryptophan could be also responsible of spinal deformities in sea bass fingerlings, as well as crystalline lens opacity and increased levels of Ca^{++} and Mg^{++} in the liver (Tibaldi and Kaushik, 2005).

However, supplementation with synthetic amino acids is not allowed according to Council Regulation (EC) No 834/2007 Article. 15 1d. (IV) and currently no amino acids are listed in Annex VI of Commission Regulation (EC) No 889/2008. Furthermore, procedures for the removal of anti-nutrients have to follow organic rules. Finally, there is less availability of relevant organic plant sources to optimize the amino acid profile in comparison to conventional plant sources (Lund et al., 2011; Rembiałkowska, 2007).

Lysine and methionine are often the most limiting amino acids when fish meal is replaced by plant protein sources (Mai et al., 2006). The amino acids which are in excess when the first limiting amino acid runs out will be broken down producing energy and nitrogen (mainly excreted as ammonia with potential adverse environmental impacts) instead of being converted to fish meat. Therefore, a carefully balanced amino acid profile is important for the growth of the fish, as well as the minimization of nitrogen discharge. To some extent the unfavorable amino acid composition in plant proteins can be balanced by combining different ingredients. However, in some cases the available organic feed ingredients will not provide a balanced amino acid profile, and in these cases the use of supplemental amino acid sources should be considered, cf. Reg 834/07; art 15 1(d).

Experiments with plant proteins (soybean, rapeseed, corn gluten, wheat gluten, pea and lupin meals) have shown potential replacement of fish meal with up to 25 to 35% (Negas and Alexis, 1995; Pereira and Oliva-Teles, 2003; Lanari and D'Agaro, 2005; Hardy 2010; Enami 2011). In sea bream it was observed that diets containing high levels (no more than 75%) of plant ingredients (corn gluten meal, wheat gluten, extruded peas, rapeseed meal and extruded whole wheat) did not affect fish growth performance and had minor effects on quality traits of marketable fish (De Francesco et al., 2007). The feed ration may comprise a maximum of 60% of organic plant products (Commission Regulation (EC) No 889/2008, Article 25(k)(3).

The most important shrimp species in aquaculture are the white shrimp (*Litopenaeus vannamei*), and the giant tiger shrimp (*Penaeus monodon*). Although they are benthivore species, they have different diets in their natural habitats, the white shrimp is an omnivorous benthivore that mainly feeds on living preys and detritus (FAO 2011), whereas the giant tiger shrimp is a carnivorous benthivore that mainly feeds on worms, crustaceans and molluscs (Tacon 2002, Piedad-Pascual 1984). These differences in feeding habits are due to the amount of enzymes in the digestive tract of the different shrimps. Carnivorous shrimps have proteolytic enzymes like trypsin and chymotrypsin, whereas herbivorous species have more glucolytic enzymes like amylase. This is why carnivorous shrimp have a greater ability to digest protein and herbivorous shrimp have greater ability to digest plant material.

Although it was estimated that optimum proteins level for giant tiger shrimp is 40 to 50% (Conklin 2003; Mahmood et al., 2005), protein needs could change to sustain shrimps maturation, reproduction and offspring quality (Wouters et al., 2001). Furthermore, the need for protein varies among species and the life stage of the animals. Younger stages have higher needs than older stages (sub-adults and adults), due to the different growth rate (Weir 1998). According to the available scientific literature, the needs for protein are between 20 and 30% of the dry matter in feed for the white shrimp (Velasco et al. 2000; Cruz-Suarez et al. 2000; Kureshy and Davis 2002), and between 35 and 50% of the dry matter in feed for the giant tiger shrimp (Fox et al., 1998; Cousin, 1995; FAO 2011; Dayal et al., 2003; McVey, 1993).

Experiments with fish meal substitution were also conducted on carnivorous species of penaeids shrimps. Both partial and total substitution of fish meal with soybean meal (SBM) in the form of soy-protein-concentrate (SPC, 65% protein) were tested on giant tiger shrimp by Paripatananont et al. (2001). Generally, high dietary concentrations of soy bean products in some species of shrimp negatively affect palatability. The authors showed that up to 17.5% inclusion of SPC in shrimp feed does not adversely affect the feed intake and the growing rate, while further progressive levels of substitution lead to impair body weight gain until the severe effects showed at 100% substitution level.
Other studies were conducted to substitute fish meal and soybean meal in shrimp aquaculture with microbial floc meal, produced in sequencing batch reactors (SBRs) (Kuhn et al., 2009, Emerenciano et al., 2012). Microbial biofloc have shown favourable nutritional quality and enhanced growth and production of shrimps (Kuhn et al., 2009, Emerenciano et al., 2012). Moreover, biofloc technology (BFT) showed to create economical and environmental benefits via reduced water use, effluent discharges, artificial feed supply and improved biosecurity (Emerenciano et al., 2012).

The replacement of fish meal by vegetable proteins is further complicated in finfish species, since not only the overall dietary amino acid profile is important for efficient utilisation of amino acids, but also the timing by which amino acids from different protein sources appear in the blood stream after a meal (Larsen et al., 2012).

Larsen et al. (2012) investigated differences in amino acid up-take, i.e. plasma free amino acid concentration patterns in juvenile rainbow trout (*Oncorhynchus mykiss*) fed either a fish meal based diet (FM) or a diet (VEG) where 59% of fish meal protein (corresponding to 46% of total dietary protein) was replaced by a mixture of plant proteins from wheat, peas, field beans, sunflower and soybean. Results showed that the appearance of most amino acids (essential and non-essential) in the plasma was delayed in fish fed the VEG diet compared to those fed the FM diet. Essential and non-essential amino acids furthermore appeared more or less synchronously in the plasma in fish fed the FM diet, while the appearance was less synchronised in fish fed the VEG diet. Further there were 2.7 times more indigestible carbohydrates in the VEG diet than in the FM diet, which suggested that the uptake of amino acids was affected by dietary carbohydrates. In conclusion, the study showed that amino acid uptake patterns were affected when replacing fish meal with plant based protein ingredients.

According to Reg. 889/2008 Article 25k fish meal and fish oil from trimmings is prioritized as ingredient for feed for aquaculture animals. According to Reg. 889/2008 Article 25k(b) fish meal and fish oil from organic aquaculture trimmings is prioritized followed by Reg. 889/2008 Article 25k(c), which states fish meal and fish oil and ingredients of fish origin derived from trimmings of fish already caught for human consumption in sustainable fisheries as the next option.

However, using fish meal from trimmings in fish feed imply, as well potential nutritional as environmental concerns. Fish meal derived from trimmings might conflict with national environmental legislations due to too high phosphorus concentrations. Fish meal from trimmings is lower in protein and higher in phosphorus content compared with high quality fish meal (Eurofins; www.ffskagen.dk). The presence of carcass remnants (head, skin, bones) in trimmings also increases the phosphorus content of the fish meal. Using this meal for feeding fish puts limitations on the inclusion level so as to comply with environmental legislation. For instance, the Danish environmental legislation only allows the phosphorus content of fish feed to be max. 0.9% (max. 1% on dry weight basis) (www.retsinformation.dk/Forms/R0710.aspx?id=140333), while no lower limit exists in for instance Norway.

There are different chemical forms of phosphorus in the diet. Highly significant differences were observed on the digestibility of the various forms (bone, phytin or organic phosphorus). Other factors, such as particle size and feed processing techniques are also known to affect its digestibility (Azevedo et al., 1998). Nonetheless, the use of phytase in fish feeds can help to reduce phosphorus waste (Lazzari and Baldisserotto, 2008). Higher phytase levels in the feed was found to increase phosphorus, as well as nitrogen bioavailability and utilization in plant-based diets used in sea bream aquaculture (Morales et al., 2013).

However, phosphorous from fish meal may have very low bioavailability, and diets with theoretically adequate or surplus phosphorous levels, can give phosphorous deficiency in salmon (Albrektsen et al., 2009).

The challenges are much higher for producing feeds for organic aquaculture because the list of available ingredients is limited and supplementation with synthetic amino acids is not allowed according to Council Regulation (EC) No 834/2007 Article 15(1)(d) (IV) and currently no amino acids are listed in Annex VI of Commission Regulation (EC) No 889/2008.

Fish meal and fish oil from organic aquaculture trimmings are also not allowed in the feed for aquaculture animals of the same species. As a result, only limited quantities of trimmings from organic farming are available. The current organic fish production (excluding shellfish and others) is about 25,000 t (Zubiaurre, 2013). About 50% of this is sold as whole fish from the farm itself, fish shops etc. and the remaining 50% (around 12,500 t) is processed into fillets, yielding about 50-60%, leaving about 40 to 50% trimmings. The amount of trimmings available for manufacturing of fish meal and fish oil may therefore be about 5,000 to 6,000 t. When we assume a yield of fish meal and oil of max. 20% and 6% respectively, this means a production of approximately 1,000 t of fish meal and 300 t of fish oil. Taking the needs of different species into account, these amounts are only sufficient for a very limited organic production and are below the critical level needed for sustainable manufacturing processes.

The manufacturing process to obtain fish meal and oil from trimmings is similar to that of wild caught industrial fish (sand eel, blue whiting etc.). However, due to the carcass remnants and the little remaining meat, the protein content of the meal from trimmings is 67–70% and the ash content is about 15%. Further, the digestibility is below 90% (pers. comm. Klaus Christoffersen, FF, Skagen, Denmark), whilst it should be at least 90% in a high quality fish meal.

Carnivorous fish require relative high dietary protein content of the diet, depending on fish size, with the highest requirement and quality for fry and brood-stock. The optimum protein level in the diets for sea bass juveniles was estimated to be around 50% (Hidalgo and Alliot, 1988; Peres and Oliva-Teles, 1999), independently from water temperature, while optimum protein level in the diet for gilthead sea bream fingerlings is around 51% at 25°C and 46% at 10-14°C (Fountoulaki et al., 2005). This means that, to produce an adequate feed, the inclusion rate of fish meal from trimmings should be high, which conflicts with the limitations of max. 0.9% dietary phosphorus content. Furthermore, the available organic plant sources are limited and their amino acid profiles are not adequately balanced to make an optimum fish feed (Lund et al., 2011). The breakdown of surplus amino acids is likely to result in increased environmental impact and reduced growth, health and welfare of the fish.

There are several other potential feed ingredients in addition to the plant proteins, such as microbial organisms (bacteria, fungi, microalgae), terrestrial animal by-products (PAP, blood meal) wild-harvested and/or cultured annelid worms, insect larvae/pupae, gastropods (e.g. golden apple snail) which may also be candidates to replace fish meal in aquaculture feed in the future (Bergleiter et al. 2009; Sørensen et al., 2011).

Microbial ingredients, i.e. products from bacteria, yeast and microalgae, are expected to have an important potential in future feeds for salmonids, sea bass and sea bream. A special aspect of some of these products is that they can be produced with different kinds of waste as raw material, and thus contribute to recycling of valuable nutrients. A large number of products, produced from various single cell organisms grown on different materials, have been investigated (Anupama and Ravindra, 2000; El-Nawwi and El-Kader, 1996; Mathews et al., 2011; Rajoka et al., 2006). Depending on type of organism, the proximate composition and amino acid profile can be much similar to fish meal (Øverland et al., 2010). A number of products have been tested as protein sources in fish feeds, and the suitability varies among the different products, inclusion levels and fish species tested (Oliva-Teles and Gonçalves, 2001; Li and Gatlin, 2003; Berge et al., 2005; Aas et al., 2006; Palmegiano et al., 2009; Romarheim et al, 2011; Øverland et al., 2013).

Microalgae as raw matter or a feed ingredient for fish have also gained interest, as they are the natural start of the food chain in the oceans. Microalgae are fed on by zoo-plankton, which again is fed on by fish. The idea is to harvest from the first trophic level or cultivate in closed system and provide feed ingredients for farmed fish by culturing microalgae. Although the benefit of different micro algae in an organic feed use have to be demonstrated scientifically for each aquaculture species, a mass production and an economic model have to be developed.

Living micro-algae are used in aquaculture for fish feeding during the early stages and the benefit of marine *Isochrysis. sp* addition in cultivated zooplankton for sea bass was demonstrated on immune and digestive fish system (Cahu et al., 1998). Modern process and algae cultivation in photo-bioreactor or fermentation system can provide algae under a flour form which can be used with the same form as fish meal for the production of formulated pellets. The chemical composition of micro-algae varies depending on species, cultivation parameters and the potential as a feed ingredient varies accordingly (Skrede et al., 2011). The microalga T-Iso (*Isochrysis spp*.) nutrients support gilthead sea bream juveniles growth better than control diets, and the chemical composition of sea bream fillets also meets the needs of consumers, although level of proteins is low compared to conventional diet and level of fats is higher. T-Iso resulted highly digestible, and supported the best performances of fish fed on a diet based on 70% of microalgae, probably due to its high protein efficiency (Palmegiano et al., 2009).

Algal addition in nutritional assay has been conducted with rainbow trout fry using a biomass of photosynthetic micro-organisms composed by a mixture of *Scenedesmus* sp. and *Chlamydomonas* (29.6% of crude protein) from a fish farm sedimentation pond. The results obtained show that a maximum of 12.5% of algal biomass can be incorporated in the feed for rainbow trout fry without negative consequences on growth and body content in lipids and energy of fish (Dallaire et al., 2007).

The evaluation of microalgae *Isochrysis sp*. in partial substitution of fish meal in gilthead Sea bream pellets showed better performances than control diets. The best performances of fish fed on 70% algae diet was probably due to the proteins composition and the amino acid profile in comparison to other diets (Palmegiano et al., 2009). Other algae species as *Tetraselmis suecica* was able to replace up to 20% of European sea bass protein without hampering growth performance and major quality traits fish (Tulli et al., 2012).

Processed Animal Protein (PAP) is an important ingredient in feeds and provides a valuable source of animal by-product utilization. Nutritional quality of rendered animal protein ingredients is affected by composition, freshness of raw materials, and processing conditions. PAP has a high nutritional value making it an excellent alternative to imported proteins such as soya. It has a significantly higher protein value (45-90% on a fed basis) than plant feed ingredients. PAP contains 10% phosphorus, which is low in relation to the content of amino acids. Blood meal is also a feed ingredient with high protein content (80% in full blood) and excellent protein digestibility (Bureau et al., 1999). It has high content of lysine and histidine, while the content of isoleucine is low (El-Haroun and Bureau, 2007; Breck et al., 2003). While there may be consumer and producer concerns about the feeding of PAP to fish, due to the potential transmission of prions, the scientific panel opinion published by the European Food Safety Authority (EFSA) in 2011 concluded that processed animal protein in feed for food producing non-ruminants, respecting the proposed ban on intra-species recycling, presents a negligible risk to human health (EFSA, 2011).

The use of insects as a source of protein in fish diets is also being explored. The chemical composition of prepupae larvae varies with species, age, method of processing and the substrate the maggot is produced on (St-Hilaire et al., 2007a,b; Aniebo and Owen, 2010). The nutritive value of insects as feeds for fish, poultry and pigs has been recognised for some time in China, where studies have demonstrated that insect-based diets are cheaper alternatives to those based on fish meal. The insects used are the pupae of silkworms (*Bombyx mori*), the larvae and pupae of house flies (*Musca domestica*) and the larvae of the mealworm beetle, *Tenebrio molitor*. Silkworm pupae are an important component of cultured cyprinid diets in Japan and China (for further information on carp and other fresh water species see section 2.4). Dried ground soldier fly larvae have been fed to chickens and pigs with no detrimental effects (Newton et al., 1977; Hale, 1973). In recent years there has been some interest in the use of housefly maggot meal as a substitute for fish meal in tilapia (*Oreochromis sp.*) and African catfish (*Clarias gariepinus*) diets (Adesulu and Mustapha, 2000; Fasakin et al., 2003; Ajani et al., 2004; Ogunji et al., 2006). Bondari and Shepherd (1987) observed that channel catfish (*Ictalurus punctatus*) and blue tilapia (*O. aureus*) fed on soldier fly larvae for 10 weeks were acceptable as food by consumers. Growth and organoleptic quality were not affected when common carp were fed on non-defatted silkworm pupae, a major by-product of the sericulture industry in India (Nandeesha et al., 2000). Ng et al. (2001) demonstrated that *T. molitor* larvae meal was highly palatable to the African catfish and could replace up to 40% of the fish meal component without reducing growth performance.

St-Hilaire et al. (2007) describe a study in which they determined if black soldier fly (*Hermetia illucens*) pre-pupae and housefly pupae could be used as a partial replacement for fish meal and fish oil in rainbow trout diets. Their data suggest that a rainbow trout diet in which black soldier fly pre-pupae or housefly pupae constitute 15% of the total protein has no adverse effect on feed conversion efficiency over a 9-week feeding period. However, rainbow trout fed on black soldier fly diets low in fish oil had reduced levels of omega-3-fatty acids in the muscle. According to the researchers, modifying the diet of the fly larvae could improve digestibility and fatty acid content of the pre-pupae, which in turn could enhance the fatty acid profile of the fish fed on the fly pre-pupae. The use of the black soldier fly in manure management, yields abundant numbers of fly pre-pupae. The authors of the study suggest that fly pre-pupae may be an economical and sustainable feed ingredient for carnivorous fish diets. However, before fly pre-pupae can be used commercially in rainbow trout diets, a larger trial over a longer period should be conducted to confirm their preliminary results. The CABI abstracts (www.cabi.org/) database contains some 700 records describing research on alternative protein sources for use in aquafeeds.

2.1.3. Gaps between organic regulation – scientific and industrial standards

Feed for organic fish is currently produced according to the EU regulations 834/2007, 889/2008 and 710/2009. However, the transitional period for exemptions is ending by

31.12.2014. It will be challenging for the feed industry to source ingredients strictly according to the regulation.

Related to the regulation 889/2008, Art. 25k on sourcing fish meal and fish oil from trimmings, it has to be considered that the levels of phosphorus in the fish meal derived from trimmings might conflict with national environmental legislations, because this may result in too high phosphorus concentrations. Fish meal from trimmings is lower in protein and higher in phosphorus content compared with high quality fish meal. The presence of carcass remnants (head, skin, bones) in trimmings also increases the phosphorus content of the fish meal. Using this meal for feeding fish puts limitations on the inclusion level so as to comply with national environmental legislation.

It means that to comply with the environmental legislation, a lower inclusion level of trimming-fish meal is requested for feed for organic fish. However, the protein content of trimming-fish meal is lower than in conventional fish meal, which stresses the need for balancing the amino acid profile of the feed. For conventional feeds a long list of alternatives protein sources exists, as well as the diets can be balanced by supplementing free amino acids, which is currently not allowed in organic aquaculture.

However, the overall environmental impact of using trimmings for sourcing protein and oil for aquaculture feed may be question marked. A Life Cycle Analyses (LCA) comparing certified organic to conventional feed for Atlantic salmon (Pelletier and Tyedmers, 2007) showed that using fisheries by-products instead of fish meals and oils from reduction fisheries increased the environmental impact, cf. section 2.5. Hence, the current regulation requesting sourcing ingredients among organic crop products and fish oil and meal derived from trimmings seemed to fail the aim of reducing the environmental impact of organic aquaculture production.

Hence, the challenges are much higher for producing feeds for organic aquaculture because the list of available alternative ingredients is limited and as previously mentioned supplementation with synthetic amino acids is not allowed according to current regulation for organic aquaculture. Farmed fish need a balanced dietary amino acid profile and especially the essential amino acids have to be provided in the diet in specific proportions. If this is not the case the surplus amino acids will be burned off and the result is compromised fish welfare and environmental impact conflicting the organic principles.

It is a fact that fish meal and fish oil are important components of aquaculture fish diets, particularly for carnivorous fish and crustaceans, which have specific amino acid and other nutritional requirements. It is important for fish welfare, quality and environmental considerations that the diet for carnivorous fish and crustaceans includes fish meal derived from whole fish, in particular feed for fry and brood-stock, but also for on-growing fish, until sufficient alternative organic sources of proteins/amino acids and oils/fatty acids are available.

For penaeid shrimps, considering a semi-intensive farming in ponds, it seems difficult to meet the animal’s nutritional requirements considering the limitation of 10% fish meal in the feed ration (Reg. EC 889/2008, art. 25l, par. 3).

It is obvious that a carefully balanced amino acid profile is important for the performance of the fish, as well as the minimization of nitrogen discharge. To some extent the unfavorable amino acid composition in plant proteins can be balanced by combining different ingredients. However, in some cases the available organic feed ingredients will not provide a balanced amino acid profile, and in these cases the use of supplemental amino acid sources should be considered, cf. Reg 834/07; art 15 1(d).

2.1.4. Conclusions and research gaps

- Sourcing of feed ingredients for organic aquaculture should be re-considered and supported by experimental data to secure compliance with organic principles of fish welfare and environmental sustainability.
- At least until more knowledge is available, fish meal and fish oil derived from industrial fish caught in sustainable fisheries, should be considered as ingredients in feed for organic carnivorous species. This includes feed for fry and brood-stock, as well as for on-growing fish, until sufficient alternative sources of protein and oil are available.
- The use of other alternative feed ingredients providing high content of essential amino acids and lipids, where possible produced organically, may be considered to be used in priority to purified or free amino acids as feed supplements/additives.
- If not available from organic procedures, essential amino acids and lipids obtained by fermentation or other similar procedures more close to the organic principles should be considered.
- Studies have indicated that not only the overall dietary amino acid profile is important for efficient utilization of amino acids, but also the timing by which amino acids from different protein sources appear in the blood stream after a meal. A significantly higher amount of indigestible carbohydrates have been measured in a diet based on vegetables than in a fish meal based diet, which suggested that the uptake of amino acids was affected by dietary carbohydrates. This issue also needs attention.
- Procedures in compliance with organic rules for removal of anti-nutrients in plant sources.
- Development of relevant organic plant sources to optimize the amino acid profile by mixing the protein sources and hence produce an optimum balanced diet for organic fish.

2.2. Fish Oil Replacement

Fish oil is a major natural source of the long chain omega-3 HUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can be synthesized by salmonids and other marine species only at a limited rate, and thus are required in the diet. Omega-3 HUFAs are produced by marine phyto- and zooplankton, which are consumed by the wild marine fish larvae (Baron et al., 2013). Hence, fish meal and fish oil are strategic ingredients to be used at critical stages of the life-cycle, when optimum performance is required.

2.2.1. Regulations

According to Commission Regulation (EC) No. 889/2008; Art. 25k, the feed ingredients (mainly fish meal and – oil) shall be sourced in priority. See also Art. 25l, par. 3.
More in detail cf. 2.1.1.

2.2.2. Current scientific knowledge

Atlantic salmon and rainbow trout are capable of converting ALA to EPA and DHA, but the conversion is not very efficient (Ruyter et al., 1999, 2000a, b, c, Tocher et al., 2000; Bell et al., 2001; Bell and Dick, 2004). Therefore their essential FA requirements are not met by C18 FAs alone, and must be provided with dietary EPA and DHA in order to obtain good growth and health (Ruyter et al., 2000a, b, c). It is also shown that the ability to convert 18:3 n-3 to EPA and DHA is induced by plant oil inclusion in fish diets (Moya-Falcon et al., 2005), and that the conversion is higher in the freshwater stage prior to smoltification, than at later post smolt life stages in seawater (reviewed by Bell et al., 2011).

Bell and Dick (2004) showed that DHA synthesis was at its highest in rainbow trout in the period immediately after start feeding, and then declined over a period of a few weeks. It has also been shown that the capacity for conversion of ALA to EPA and DHA, and the gene expression of the Δ5- and Δ6- desaturase activities in salmon is depressed when fed high dietary levels of FO, while vegetable oils to a certain degree increased the capacities (Ruyter et al., 2003, Moya Falcon et al., 2005, Kjær et al., 2008). Increased content of 18:3n-3 in feed will not necessarily lead to increased conversion to EPA and DHA, because there is competition for Δ6- desaturase from two steps in the pathway. Too much 18:3n-3 may hamper further metabolism to EPA and DHA (Ruyter et al., 2000a).
Several studies have shown that there are species differences in the capacities for conversion of essential FAs (Sargent et al., 2002). It is well known that rainbow trout can synthesize DHA from ALA (Buzzi et al., 1996; Bell et al., 2001; 2004), and this process is also taking place when fish are fed diets high in 22:6n-3 (Buzzi et al., 1996). The studies indicate that rainbow trout has better ability than Atlantic salmon to convert the shorter vegetable n-3 FAs to the important long-chain EPA and DHA.

Diets based on vegetable oils have shown generally good growth results in salmon, but with major challenges in the body lipid composition (Thomassen and Røsjø, 1989; Sargent et al., 2002; Grisdale-Helland et al., 2002; Torstensen et al., 2005). Altered FA composition may affect the fish health. Omega-3 fatty acids have important biological functions in the fish (Montero et al., 2010; Torstensen et al., 2013), and a change in dietary fatty acid composition is expected to affect fish performance and health. The omega-3 fatty acids serve as the building blocks of cell membranes, regulate gene expression, and are precursors of a range of bioactive substances that regulate inflammation, physiology and satiation. By optimizing dietary fatty acid composition, the retention of EPA+DHA can be optimized and thereby improving fish health as well as securing the farmed salmon as a good source of EPA+DHA for human consumption.

The balance between n-6 and n-3 FAs in the diet seems to be important, as the pro-inflammatory eicosanoids from the n-6 family are more abundant and have greater bio-potency than their n-3 homologues (Lands 1992). This may have impact on several aspects of fish health. 18:2n-6 and 18:3n-3 also compete for the same enzyme systems, for synthesis of long chain PUFAs. The optimal n6/n3 ratio for conversion of 18:3n-3 to EPA and DHA in salmonids is not known.

The feed oils for the future may consist of a mix of different oils that provide an optimal dietary ratio between groups of FAs (C18 n-6 / C18 n-3 / C20 n-3 / C22 n-3), and utilises the innate ability of the fish to produce EPA and DHA. The optimal mix will be defined by the trade-off between high rate of deposition (retention) of dietary long chain n-3 HUFAs and high absolute level of these fatty acids, which are to some extent conflicting aspects. The optimal oil mix (FA combination) may vary for different life stages and in different environments.

Salmonids prior to smoltification have higher Δ-5 and Δ-6 desaturase activities than fish in seawater (Sargent et al., 2002). Oils that contain appropriate levels of C18 n-3 fatty acids, but relatively low content of C18:2 n-6 are wanted. Linseed oil, as well as less commonly used oils like camelina oil and chia oil are candidates that are rich in C18 n-3. One must however be aware that it is shown that high dietary levels of ALA seem to inhibit its own conversion to DHA in Atlantic salmon, and the dietary level must therefore be optimised (Ruyter et al., 2000a, b). Camelina oil has recently been tested in diets for Atlantic salmon (Hixon et al., 2014), and 100% exchange of fish oil with camelina oil caused a small but not significant drop in growth rate. Salmon lipid composition reflected the dietary fatty acid profile, with a higher content of 18:3 n-3 in fish fed the camelina oil diet. Echium (*Echium plantagineum*) oil is another promising oil, with high content of C18:4n-3, one step further to a long chain n-3 FA compared to 18:3n-3. Echium oil has been tested in diets for Atlantic salmon (Codabaccus et al., 2010), and higher biosynthesis of eicosatetraenoic (20:4n-3) and 20:5n-3 was indicated in the Echium oil group compared to FO and canola oil groups.

For the replacement of fish oil, marine fish species as sea bass and sea bream (Geay et al., 2011) have lower tolerance to vegetable oil compared to freshwater or anadromous fish species such as salmonids. This lower adaptation of marine fish species to vegetable oil can be linked to their lower efficiency in synthesizing LC-PUFA from n-3 and n-6 precursors present in plants (Geay et al., 2011). A high or total substitution of fish oil by plant oils induced decreases in growth rate of gilthead sea bream and European sea bass (Geay et al., 2010; Montero et al., 2010). Studies on sea bream showed that a replacement up to 66% of fish oil can be operated by a vegetable source with comparable results. Nutrient absorption in fish intestine was negatively modified for a total substitution of fish oil by vegetable oil. An impaired digestion was observed induced by an accumulation of lipidic droplet in the fish intestine (Santigosa et al., 2011a) probably due to a saturation of fish assimilation sites. Indeed, LC-PUFA, used as structural components of cell membranes, are also the principal precursors of eicosanoids, that are involved in many physiological processes such as osmoregulation, immune responses, blood coagulation and reproduction (Bell et al., 1997; Geay et al., 2011).

The lower nutritional value in the flesh of marine fish fed vegetable diet is generally due to the low content in EPA end DHA. *Isochrysis* sp., partially substituted in sea bream diets, showed to be a good source of polyunsaturated fatty acids and in particular of docosahexaenoic acid (DHA) (Palmegiano et al., 2009).

Progressive substitutions of fish oil with Cottonseed Oil (CSO) do not affect fish growth, feed conversion ratio and protein utilization, but hepatosomatic and visceral fat indexes increased with increasing dietary CSO (Eroldogan et al., 2012). CSO, being a rich source of n-6 PUFA, may affect hepatocyte vacuolation and lipid infiltration, and this could be likely ascribable to the reported lipogenic effect of 18:2n-6, as suggested by Montero and Izquierdo, (2010).

Partial substitution (50%) of fish oil respectively with sesame oil (SO), canola oil (CO) and soybean oil (SBO) in sea bass did not influence the whole body fatty acid composition in terms of saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) contents (Özşahinoğlu et al., 2013). The diet that showed the best growth performances was the one using sesame oil as substitute. Also the partial substitution (50%) of fish oil with soy bean oil in both sea bass and rainbow trout had no effects on either hepatic lipid droplets accumulation or the degree and pattern of vacuolization (Figueiredo-Silva et al., 2004).

Higher levels of fish oil substitution was applied in both sea bass and sea bream by Izquierdo et al. (2003) using respectively soya bean oil (SO), rapeseed oil (RO) and linseed oil (LO) or a mixture (Mix) of these. Feed intake was not influenced by the vegetable oils, as well as fish growth. Fatty acid composition of liver and muscle reflected that of each single diet, but utilization of dietary lipids differed between these two tissues and was also different for the different fatty acids. In particular, sea bass liver showed much higher lipid contents than sea bream, due to a greater accumulation of saturated (mainly 16:0) and monounsaturated (mainly 18:1n-9) FAs. Muscle lipid contents were very similar for both species.

Mourente et al. (2005) showed that vegetable oils such as rapeseed, linseed and olive oil can potentially be used as partial substitutes for dietary FO in European sea bass culture, during the grow-out phase, without compromising growth rates, but may alter some immune parameters. Indeed an alteration of the non-specific immune function was observed and the number of circulating leucocytes was significantly affected, as well as the macrophage respiratory burst activity. Accumulation of large amounts of lipid droplets were observed within the hepatocytes in relation to decreased levels of dietary n-3 HUFA, although no signs of cellular necrosis were evident. Inclusion of vegetal oils (rapeseed, linseed and olive oil), up to 600 g kg^{-1} of dietary oil, significantly reduces EPA and DHA and increases linoleic and linolenic presence in sea bass flesh. The time required to restore individual fatty acids to values similar to those in fish fed fish oil were different for each fatty acid. In the same study Mourente et al. (2005) also observed that some fatty acids are selectively retained or utilized. In particular, there is a selective deposition and retention of DHA because flesh DHA concentrations were always higher than diet concentrations, as observed also for salmonids. Linolenic (LNA; 18:3n-3), linoleic (LA; 18:2n-6) and oleic (OA; 18:1n-9) acids concentration significantly increase in flesh lipids following the fish oil substitution with vegetable oils. This should be taken into account, as reducing n-6 PUFA, largely as linoleic acid, has benefit implication in the human diets.

Typically, crustaceans have limited ability to *ex novo* synthesize HUFA, as observed in marine fish (Mourente, 1996), at least at the beginning of maturation. Similarly there are difficulties for the *ex novo* synthesis of cholesterol (Kanazawa et al., 1988), useful to synthetize steroid hormones (Kontara et al., 1997). Lipids are essential components of the diet of shrimps and are mainly used for direct energy production and cell membrane building. For the giant tiger shrimp and the white shrimp the optimal lipid level is between 6 and 8% of the feed dry matter (Alday Sanz 2011; Tiwari and Sahu, 1999), but should not be above 10% (Glencross 2002) or below 2% (Chen, 1998).

Some lipids are more important than others since they cannot be synthesized *de novo* or not in sufficient amounts by shrimps. Phospholipids (e.g. lecithin) and cholesterol are the two main categories of essential lipids for shrimps. They are also used as emulsifiers for lipid digestion. Without phospholipids in their diet, shrimps are unable to digest lipids properly.

According to the available scientific literature, the need for phospholipids is for giant tiger shrimps is 1% of the diet for post-larvae (Paibulkichakul et al., 1998) and 1.25% for juveniles (Chen 1993). In addition, for the white shrimp, the requirements for lecithin and cholesterol are linked together.

Cholesterol is a ring compound, which is part of cell membranes and is also necessary in the moulting process. According to the literature, the need for cholesterol varies among the different species of shrimps and according to the different life stages. For giant tiger shrimps, cholesterol need is crucial and cannot be replaced. Requirements are 1% of the diet for post-larvae (Paibulkichakul et al., 1998) and 0.17% of the diet for juveniles (Smith et al., 2001). For the white shrimp, there is a relationship between cholesterol and phospholipids. A diet with no phospholipids requires 0.35% cholesterol, whereas a diet with 5% phospholipids requires only 0.05% cholesterol (Gong et al., 2000). A good combination seems to be 0.15% of cholesterol for 1% or more phospholipids.

Micro-algae and especially marine species are promising alternative fatty acid sources of interest in aquaculture feed. It was shown that microalgae oils from *Isochrysis, Nannochloropsis, Phaeodactylum, Pavlova* and *Thalassiosira* contain sufficient omega-3 LC-PUFA to serve as an alternative for fish oil (Ryckebosch et al., 2014).

Evaluation of microalga *Isochrysis.sp* in partial substitution of fish meal revealed a positive effect on gilthead sea bream performances and chemical composition of fillets. Best fish performances was observed when fish fed on 70% algae diet, probably due to highest amount of saturated fatty acids, mainly due to myristate and palmitate acid (Palmegiano et al., 2009). The use of heterotrophic algae source *Schizochytrium* or *Crypthecodinium cohnii* in the early sea bream stage showed an important potential of these stains as alternative DHA sources for fish feed in microdiets and also point out the necessity of EPA sources to completely replace fisheries-derived oils (Atalah et al., 2007; Ganuza et al., 2008).

2.2.3. Gaps between organic regulation – scientific and industrial standards

Conflict between omega-3 fatty acid (highly unsaturated fatty acids – HUFAs) content in fish (human health) and the regulation requesting exchange of fish oil with plant oil mostly low

in omega-3 fatty acids. The promotion of (organic) fish as healthy is impeded. Need of development of plant oils high in omega-3 fatty acids. However, non-GMO plants are not able to produce omega-3 fatty acids longer than 18C, and can not supply EPA and DHA.
Possible conflict with the organic regulation in relation to the use of cholesterol and lecithin as feed additives.

2.2.4. Conclusions and research gaps

- It is important to keep focus on human health related to consuming (organic) aquaculture products, including high content of long chain omega-3 fatty acids (EPA and DHA) currently sourced from fish oil.
- Adjust regulation on request of exchanging fish oil by vegetable oils in accordance to development of vegetable or other sources producing omega-3 fatty acids (HUFAs).
- Priority research in alternative sources of Omega-3 fatty acids (HUFAs).
- The use of cholesterol as raw material in the feed for supplementing the diet of shrimps is in line with the objectives and principles of organic production and should be allowed.
- For preference, lecithin from organically certified sources, such as organic soybean, may be used following mechanical extraction. If unavailable, non-organic natural sources may be used provided they are of non-GMO origin.

2.3. Mineral and vitamin supply

Information on requirements of minerals and vitamins is limited, but for salmonids the needs are still among the best documented. In addition, some knowledge is available for species such as sea bass and sea bream.

2.3.1. Regulations

Dietary supply in organic production is preferably from natural origin, but chemically well-defined analogic substances may be authorised for use if the natural substances are unavailable (EC 834/2007, rec. (16 2(e) (ii)).

2.3.2. Current scientific knowledge

There are factors that complicate the assessment of dietary requirement of minerals and vitamins. Fish may absorb some of these nutrients from the water, and nutrients may leach from diet to water, difficulties in producing good test diets, and lack of knowledge on bioavailability of the nutrients. The current practice is to add nutrients to the diet, based on existing knowledge, but with a significant safety margin. Because of the weak evidence, dietary requirements may be underestimated for some of these nutrients.

Minerals are divided in macro-minerals (P, Ca, Mg, K, Na, Cl; required in relatively large amounts) and micro minerals (Zn, Cu, Se, Mn, Mb, Fe, I, Cr, Co). Phosphorous and calcium are needed in large amounts for skeletal tissue, as well as other functions. In Atlantic salmon, skeletal deformities are seen occasionally as a consequence of mineral (P) deficiency.

There are few available data on the mineral requirements of other marine fish such as sea bass and sea bream. For sea bream, Oliva-Teles, (2000) reported a dietary phosphorus requirement around 0.75%. Bio-availability of phosphorus is highly variable among feedstuffs, and is higher in animal than in plant feedstuffs (Oliva-Teles 2000). This is due to the major proportion of phosphorus in plants being stored as phytate, which is not available to animals. Gomes da Silva and Oliva-Teles, (1998) estimated the apparent digestibility coefficients (ADC) of phosphorus for sea bass juveniles: the ADC of phosphorus of animal feedstuffs averaged 81% while that of soybean was only 38%.

Calcium and phosphorus are two of the major constituents of the inorganic portion of feed (Davis et al., 1993). Shrimp are able to absorb calcium from the water via drinking or absorption from the gills, epidermis or both. On the other hand, phosphorus concentration in natural water is generally too low, making the dietary phosphorus income essential for shrimps. Davis et al. (1993) observed in white shrimp that in absence of dietary calcium supplementation, the adequate dietary phosphorus amount is 0.34%, although the minimum level of dietary phosphorus for maximum growth of white shrimp is dependent on the calcium content in the diet. Anyway, shrimp diet containing 3% or more of calcium should be avoided. In giant tiger shrimp, Ambasankar et al. (2006) estimated that the best zoo technical performances were recorded by the diets supplemented with 1.0 and 1.5% phosphorus.

Vitamins are needed in trace amounts in the diet in order to maintain normal growth, reproduction and health. Characteristic deficiency signs are seen in mammals in the absence of vitamins, but in fish the deficiency signs are less specific. The requirements are affected by size, age, growth rate, environmental factors and nutrient interactions.
Of the water soluble vitamins, the B-complex are needed in relatively small amounts, while choline, inositol and vitamin C are needed in larger amounts. Status on vitamin requirement knowledge is reviewed by NRC (2011). Various marine microalgae stain should provide excess or adequate levels of the vitamins for aquaculture food chains (Brown et al., 1999; Coutinho et al., 2006).
Available data on vitamin requirements of sea bass and sea bream is very scarce. A dietary requirement for vitamin B6, pyridoxine, has been demonstrated in several species. Kissil et al. (1981) reported that signs of pyridoxine deficiency were manifested in sea bream as growth retardation, high mortality, poor food conversion, hyperirritability coupled with erratic swimming behaviour and degenerative changes in peripheral nerves. The authors also estimated the dietary pyridoxine level at and above which no deficiency signs appeared: 1.97 mg kg^{-1} dry diet. Bioavailability of ascorbic acid (AA) esters, such as the phosphate forms, has been found to be high in several fish. The minimum dietary ascorbic acid stable phosphate forms requirement reported in literature is in the range of 10 –20 mg of AA kg^{-1} for freshwater fish and 12.6 – 47 mg of AA kg^{-1} for some marine fish (as reviewed by Fournier et al. (2000)). Ascorbic acid is necessary for the hydroxylation of proline, leading to hydroxyproline (HyPro), which is involved in collagen synthesis. Except for some carp (*Cyprinus sp.*) and some sturgeon (*Acipenser sp.*) species, most finfish cannot synthesize AA.

Documented pathological effects of vitamin C (ascorbic acid) deficiency in sea bream are reported by Alexis et al. (1997). Such pathological signs appeared in all fish fed the vitamin C deficient diet: extensive tubular damage, glomerulonephritis, and inflammatory response of the haemopoietic tissue producing granuloma, while the gross deficiency signs observed were anorexia, scale loss, depigmentation, internal and external haemorrhages. Henrique et al. (1998) estimated that the ascorbic acid requirements for sea bream is less than 25 mg kg^{-1}. While for juvenile European sea bass, the minimum dietary AA requirement reported by Fournier et al. (2000) to maintain normal skin collagen concentration and maximal growth is 5 mg of AA kg^{-1}, apparently below the requirement of other fish, although higher levels were required based on whole body hydroxyprolin and liver ascorbic acid concentration. Kaushik et al. (1998) tested the recommendations for salmonids of NRC (1993) for vitamin requirements in sea bass. The authors confirmed the applicability of the NRC salmonids recommendations in diets for sea bass, although in semi-purified diets a slightly higher supply was necessary to allow satisfactory growth rates. Among natural antioxidants, vitamin E has been found to offer a protective role against the adverse effects of reactive oxygen and other free radicals. Gatta et al. (2000) demonstrated that a level of 942 mg kg^{-1} in the diet is enough for sea bass.

Vitamins have pivotal roles for ensure good survival rates in aquaculture also in shrimps dietary. Indeed, vitamin C deficient diets in white shrimp resulted in biological membranes, where it contributes to membrane stability (He and Lawrence, 1993b). Moreover, it protects cellular structures against oxidative damages from oxygen free radicals and reactive products of lipid peroxidation. Lee and Shiau (2004) demonstrated that a level of 85-89 mg kg^{-1} of vitamin E is required for maximal growth and non-specific immune responses of giant tiger shrimp, while 179 mg kg^{-1} of vitamin E is required to maximise tissue vitamin E concentration.

Astaxanthin is a pigment substance that is found in the natural diet for salmon, causing the red colour of the muscle. It is also known as a potent antioxidant. The carotenoids are mobilized from muscle to skin and ovaries in maturing fish, but the role in reproduction is not fully understood. According to Torrissen and Christiansen, (1995), dietary carotenoids are required in fish diets, suggestively with a metabolic role similar to that of vitamin E and A. Astaxanthin is the preferred carotenoid for pigmentation in salmonids, and is found naturally in potential feed ingredients like shrimp, krill, calanus, capelin oil and some yeasts and algae. Astaxanthin from micro-algae, mainly extract by a green microalgae *Haematococcus pluvialis*, attract considerable attention for its biological properties such as the antioxidant activity, colouring agent and lipid sources for farmed fish feed (Choubert et al., 2006; Fujii et al., 2006). Main supply for salmonid culture is synthetic astaxanthin. An Eco-efficiency study published by BASF (Gensch et al., 2004), indicated that the sustainability in production of astaxanthin for pigmentation of salmon was best in synthetic production, and poorer in yeast and algae products. The factors considered were surface use, energy use, emissions, raw material use, risk potential and toxicity potential.

The pigmentation of shrimps, as well as for salmonids, is influenced by astaxanthin dietary intake. Indeed, an optimal pigmentation in giant tiger shrimp is guaranteed by dietary levels of 50 mg kg^{-1} of astaxanthin (Menasveta et al., 1993). Also survival and growth rates of post-larvae increase according to dietary astaxanthin in giant tiger shrimp (Merchie et al., 1998) and in white shrimp up to supplementation levels of 200 and 400 mg kg^{-1} (Niu et al., 2009). reduced survival rates, while growth was not affected (He and Lawrence, 1993a). Moreover, it was observed that whole-body ascorbic acid content in shrimp increased as dietary vitamin C increased. He and Lawrence, (1993a) estimated also that the minimum dietary vitamin C levels required for normal survival of white shrimp specimens of 0.1 g and 0.5 g are respectively 120 mg ascorbic acid-equivalent (AAE) kg^{-1} and 90 mg AAE kg^{-1}, showing that dietary vitamin C requirement of white shrimp decreased with increased size. Furthermore, there is evidence that dietary ascorbate enhances immune responses in white shrimp (Lee and Shiau, 2002).

2.3.3 Gaps between organic regulation – scientific and industrial standards

Feed for organic fish is currently produced according to the EU regulations 834/2007, 889/2008 and 710/2009. However, challenging to source minerals and vitamins from natural origin.

Possible gaps between request in the regulation of using substances of organic/natural origin and fulfillment of the requirements of the aquaculture animals, in order to secure animal health and welfare.

2.3.4 Conclusions and research gaps

Chemically well-defined analogic substances of minerals and vitamins may be authorised for use if the natural substances are unavailable.

3. State of the art freshwater carp ponds

Carp ponds can be stocked with one or with more than one fish species. The first stocking method is monoculture, while the second one is the polyculture. It is a general rule that more fish can be produced in the same waterbody if many fish species, different in their feeding habit and other biological features, are cultured together. This is because the utilization of natural food is much more efficient by a multispecies fish stock. Furthermore, if the species composition of fish is properly established and maintained.

3.1. General feeding

The production of plankton is stimulated by its intensive consumption by the fish. Synergic effects between some species may also support the higher fish production in polycultural ponds. For example, the production of common carp can be higher if reasonable quantities of silver carp and grass carps are in the same pond (Woynarovich et al., 2010).

Monoculture of fish can utilize natural fish food less effectively than polyculture. Consequently, unless very low stocking densities are applied, the production of fish in monoculture is much more feed dependent than in polyculture. Monoculture of common carp is widely practiced in many geographic regions of Central and Eastern Europe.

Nevertheless, the production figures of common carp are much lower in monoculture than in polyculture. The profitability of production is also less favourable in monoculture.

The bulk of the natural fish food organisms in the water column of fish ponds are the plankton. The phytoplankton and zooplankton contribute directly to the diet of the different species of the polyculture. Next to phytoplankton and zooplankton, the role of the bacterioplankton is also important in fish ponds (Adámek et al, 2014). This group of living organisms participates in the processes of both composition and decomposition. The bacterioplankton has a significant role in nitrogen fixation, nitrification, denitrification, remineralization, etc. The bacterioplankton serves directly as a food source of other planktonic organisms and their colonies are consumed directly by some of the fish.

The pond bottom is another important biotope in carp ponds, as different fish food organisms live and develop there. Moreover, the detritus and the bacteria, ciliates, etc., that are present in the sediments also serve as natural food for common carp, breams or tench. Water weeds that grow on the pond bottom serve as natural fish food for grass carp. Periphyton or biofilm is the collective name of organisms which live on the surface of the submerged objects and macrovegetation in a pond. These are bacteria, algae, moss and animals of different sizes. Though periphyton is less frequently mentioned as an important source of natural fish food, it may still provide a considerable quantity of food for some of the fish of pond polyculture and is considered as a very promising food source with respect to the purposes of organic farming (Milstein et al., 2008).

In ponds, the detritus, the colonies of bacteria, the aquatic weeds, the plankton and the terrestrial and water insects and their aquatic larvae are all natural food for the different fish species. In pond polyculture, the role of natural fish food is outstanding, since it is the source of protein in the diet of fish which otherwise would only be supplied by expensive fish meal.

3.2. Species in carp ponds

In carp pond polyculture, the individual fish species utilize various food resources for their nutrition:

- Common carp (*Cyprinus carpio*) is an omnivorous species with a wide food spectrum involving a considerable part of a secondary pond production. In the first year, the basic component of its diet is zooplankton which is possibly supplemented with phytophilous organisms, such as some larvae of chironomids and mayflies. Benthic food is consumed only to a limited extent and if it is easily available. If there is an insufficient amount of animal food, an emergency food, such as cyanobacteria, planktonic and filamentous algae, duckweed, live or dead macrophytes can be consumed. In the second and furhter years of life, carp particularly feed on macrozoobenthos (larvae of chironomids, tubificids). In conditions of a rich development of larger daphnias, these can be ingested (filtered) also by carp weighing several kilograms, i.e., three-year-old and older individuals. Nevertheless, benthic food represents a basic component of carp nutrition. If there is an insufficient amount of natural food, carp can also successfully feed on an emergency food (Adámek et al., 2003), such as bryozoans, macrophytes or detritus.
- Tench (*Tinca tinca*) is also an omnivorous species whose food demands are basically the same as carp (diet overlap even exceeds 60%, Adámek et al., 2003). Unlike carp,

tench forage for food mainly in littoral and overgrown parts of a pond which partially decreases the direct food competition between these two species. The basic diet of tench fry is zooplankton which also appears significantly in the diet of older fish together with zoobenthos and phytophilous organisms (Adámek et al., 2003). Duckweed, bryozoans, etc. are often consumed as an emergency food.

- Silver carp (*Hypohthalmichthys molitrix*) represents an important filtrator whose dominant food component is phytoplankton since the age of approximately two weeks (~ 2 cm). The size of gaps in its filtering apparatus is very small and it does not exceed the size of 20 µm even with large individuals which enables it to obtain even very small food organisms of phytoplankton as well as bacterial agglomerations. Its ability to digest phytoplankton is, however, very weak and most cells go through its digestive tract vital and intact (Jančula et al., 2008). It has even been revealed that it has stimulating effects on some species of phytoplanktonic algae and it thus supports eutrophication processes ("ichthyoeutrophication" according to Opuszyński, 1978). Digestion is considerably more successful if the phytoplankton cells are dead and damaged by decomposition. Silver carp is also able to successfully use small zooplankton and it can thus represent a significant competitor for carp and tench in large stocks (above 500 kg per hectare). Zooplankton (rotifers, nauplii of copepods, small cladocerans) represents the basis of silver carp's early diet.
- Bighead carp (*Aristichthys nobilis*) is also a typical planktonophagous species whose diet basically comprises all components of bioseston including a decomposing organic matter. With regard to the size of gaps in the filtering apparatus (~ 80 µm), it is a significant consumer of not only colonial forms of phytoplankton, but also smaller and larger zooplankton thanks to which it can compete in food with carp when the stocks are higher (above 300 kg per hectare) and it can thus lead to retardation of the carp´s growth potential. Similarly to silver carp, early ontogenetic stages feed almost only on zooplankton, later on, it changes over to filtering the main criterion of which is the size of food particles exceeding 80 µm. This size remains limiting for the entire life of bighead carp.
- Grass carp (*Ctenopharyngodon idella*) is a typical representative of phytophagous fishes and after it has reached the age of several months and the length above 5 cm it almost only feed on higher aquatic plants (Adámek and Sanh, 1981). However, in pond polycultures with supplementary feeding, it consumes preferably the feed that is provided to carp and it neglects macrophytes as its basic food component.

3.3. Dietary requirements

The dietary requirements of common carp for proteins, amino acids, lipids, fatty acids, carbohydrates, vitamins, minerals and protein-energy ratios have been investigated and summarised in several reviews (e.g. Ogino et al., 1979; Satoh, 1991; De Silva and Anderson, 1995; Kaushik, 1995; Takeuchi et al., 2002; NRC, 2011). The daily requirement of common carp for protein is about 1 g kg^{-1} body weight for maintenance and 12 g kg^{-1} body weight for maximal protein utilization (Ogino and Chen, 1973; Ogino, 1980b).

According to Takeuchi et al. (2002) the efficiency of nitrogen utilization for highest growth showed to be with a protein intake of 7 to 8 g kg^{-1} body weight per day. Similarly, recalculation of some data, from studies in which high growth rates were observed, showed that the protein requirements for optimal growth of common carp are 10 – 12 g kg^{-1} body weight per day (Satoh, 1991). Investigations on the optimal nutrient requirement of common carp have demonstrated that crude protein levels in the feed ranging from 30 to 38% appear to satisfy the fish. If sufficient digestible energy is contained in the diet, the optimal protein level can be effectively kept at 30 – 35% in the fish feed (Watanabe 1982).

Common carp requirements for aminoacids and essential aminoacids have been a topic of numerous studies. Their results can be found summarized in the reviews by Nose, (1979), Ogino, (1980) and Dabrowski, (1983).

Carp can utilize effectively both lipids and carbohydrates as energy sources, and therefore the overall digestible energy content of the diet is more important than the lipid content (Takeushi et al., 2002). It is well known that the differences in the digestive tract respectively the lower amylase activity in fish result in general in a lower digestibility of starch in fish than in terrestrial animals. However, carp can effectively use carbohydrates as an energy source (Ogino et al., 1976; Takeushi et al., 1979). Among fish, the amylase activity is higher in omnivorous fish, including carp, than in carnivorous fish. It has been found that common carp has a gut which is 1.5 – 2 times longer than his body length, which is 3 – 4 times higher ratio of intestine to body length compared to rainbow trout. It has been suggested to be the reason for the better utilization of carbohydrates by carp (FAO, 2014). According to Takeushi et al. (2002), the optimum amount of carbohydrates in the diet can be considered to be about 30–40% for common carp.

Even marginal deficiencies in some of the minerals, trace elements or vitamins lead to severe morphological deformities in addition to poor growth of carp. The qualitative and quantitative vitamin and mineral requirements have been well investigated in some studies and summarized by Satoh, (1991), Kim et al. (1998) and NRC, (2011).

As the name indicates, supplementary feeding in pond fish culture is practiced mainly as a supplement to the natural fish food. Natural fish food organisms are rich in proteins but poor in carbohydrate. Widely applied supplementary feeds are the different cereals. They are relatively poor in protein but rich in energy. The evaluation of cereals with their importance in carp supplementary feeding was performed by Jankovic et al. (2011). No significant differences have been found in the growth performance response of carp fed by various cereals (barley, wheat, triticale and rye) as carbohydrate compounds of extruded feeds (Przybyl and Mazurkiewicz, 2004). Contrarily, Urbánek et al. (2010) proved the differences between marketable carp fed various cereal diets not only regarding their growth performance. The carp supplementally fed with rye were found to have a higher fat content than those fed with triticale. However, the established average fat content values (except for maize) were at the level that indicated a high sensory quality of carp flesh during the whole growing season. More protein-rich feeds, together with cereals, are also used to supplement the natural fish food when the standing stock of fish increases by the end of the production season.

The consumption and utilization of supplementary feeds in carp pond culture depend on the species and the age of fish, as well as on the quantity and quality of the available natural fish food. Consequently, the feed conversion ration (FCR) of the supplementary feeds may vary within certain ranges. Feeds represent the most significant cost item within the market carp farming. New possibilities are currently being presented in fisheries for improvement of production efficiency of cereals by their suitable modification. Principle of such technologies is the increase of nutritional value, taste, acceptability and especially digestibility of carp feeds (Másílko and Hartvich, 2010).

The biological cycle in a fish pond is based on essential mineral nutrients, carbon dioxide and solar energy, from which organic materials are produced through photosynthesis. Most of the used mineral nutrients are the different forms of nitrogen (N) and phosphorus (P). Carbon (C) is obtained from CO_2. It is the result of bacterial decomposition of organic materials and the respiration of living organisms. The organic materials of the applied fresh manure, faeces of fish and the detritus may be directly consumed by the zooplankton, insects and their larvae and by some fish species or they are broken down to essential mineral nutrients by bacteria and return in this form to the start of the cycle. The materials needed for the establishment and maintenance of the food chains are supplied through manuring and/or fertilization. These ensure increased production of the natural fish food in the ponds. Feeding is one of the most crucial technological elements of fish rearing, which largely determines the profitability of the production since even the prices of supplementary feeds are usually high. The principles and practice of feeding developing fry are rather different compared with the feeding of elder age groups.

Feed applied when rearing advanced fry of carps should have high protein content (30 – 35%) and it should be finely ground. Special manufactured feeds for rearing fry are available ready to use. If the use of manufactured feeds for fry is not possible or feasible, farm-mixed feeds can also be used. At the start of production it should be flourlike (about 0.1 – 0.2 mm). Later, the size of the feed particles can be larger, proportional to the size of the mouth of the developing fry. Soaking of feed helps the digestion of the feed particles.

In the case of the elder age groups, only common carp, breams, tench and grass carp used to be fed with supplementary feeds. The daily quantity is recommended to be as much as is usually consumed by the fish within three to four hours. This will be about 1 – 5% of the standing stock of the common carp, depending on the actual consistency of the given feed, fish size and water temperature. Feeding should be done in the morning hours at the same locations marked with sticks or small buoys. Whether the consumption of the supplementary feed occurred should be checked in late morning or during early afternoon.

Cereals (wheat, barley, triticale) are the best supplementary feeds because they are rich in energy. It is commonly believed that grains or other feeds that are not suitable for terrestrial animals because of their inferior quality are still suitable for fish. By-products of mills or broken grains are certainly suitable for feeding fish. However, the feeds which are contaminated with fungi or are rotten or contain poisonous seeds of weeds should not be given to fish because they cause infection or inflammation of the digestive tract.

Grass carp can be fed with fresh terrestrial plants. Their daily portion should be consumed within 12 – 18 h. To prevent grass carp from feeding on supplementary feeds, green feeding should be done first in the morning, even before common carp is fed. If the daily portions of green feeds are placed into simple floating frames, the follow-up of their consumption becomes easy.

The FCR should be calculated each month, as well as at the end of the production season. It is a rule of thumb that the FCR will be less during the first half of the production season. In the second half of the production season it will be higher than its overall average. Another general rule is that the FCR of the same feed stuff of the younger fish will be lower than of the elder fish. This is because the proportion of protein-rich natural food is higher in the diet of younger fish; consequently, less supplementary feed is necessary to produce 1 kg of live weight.

By the last third of the production season the populations of the natural fish food organisms in ponds may decline both in absolute and in relative terms. This is when the standing stock of common carp is over 1 t per hectare. In this case, feeds or mixture of feeds with higher protein content will supplement the missing protein of the natural fish food.

4. Sustainable fisheries

In order to ensure the sustainability of fisheries, different systems have been introduced at different levels, such as The United Nations Convention on the Law of the sea, The FAO Code of Conduct for Responsible Fisheries, The United Nations Fish Stocks Agreement, and also different regional organizations (FAO, 2011). Recently the new Common Fishery Policy regulation (EU Reg. 1380/2013) has established that the Maximum Sustainable Yield should be reached for the target stocks and fishery should be conducted preserving also ecosystem functioning and integrity. The maximum sustainable yield (MSY) is the theoretical largest amount of fish that can be harvested from a stock over time without reduction in population size. This is the management tool that EU has committed to reach within 2020 for all commercially harvested fish stocks. There is also a number of independent organizations working on fish stock assessments and giving advice, e.g. FAO (UN Food and Agriculture Organization), that publish comprehensive statistics and information in order to provide politicians and other decision makers with facts. Scientific, Technical and Economic Committee for Fisheries (STECF) is the official scientific body of the European Commission that revise annually the assessments performed at the level of advice/management bodies. Research on fish stock assessments, management of stocks as well as advising total allowable catch (TAC) for actual fish species is carried out by governmental institutes as well as international non-governmental organizations. Examples of such organizations are ICES (The International Council for the Exploration of the sea), GFCM (General Fisheries Commission for the Mediterranean), that is instrumental in coordinating efforts by governments to effectively manage fisheries at regional level, ICCAT (International Commission for the Conservation of Atlantic Tunas), Regional Fisheries Management Organizations (RFMOs), IMARPE (Peru – Institute of Fisheries Research) and IFOP (Chile – Institute of Fisheries Research).

ICES, GFCM and ICCAT are implementing MSY or MSY agreed proxies in their advices. Advises from ICES are the basis for fisheries management in the EU, Iceland and Norway, while advices and recommendations from GFCM are the basis for the fisheries management in the Mediterranean (EU and non-EU) but national marine research institutes are also advising catch quotas and management of fish stocks for the national fisheries. There are examples where political fisheries authorities allow higher catches than recommended by ICES and other independent institutions, and also examples of the opposite. The European Commission is a GFCM partner, as well as the 23 Mediterranean Countries.

Private standards and certification schemes are developed to contribute to sustainability and responsible fisheries management (FAO, 2011). The international fish meal and fish oil organization (IFFO), who represents the fish meal and fish oil producers, have developed their IFFO-RS standard for Responsible Sourcing of raw materials (IFFO, 2010), and an increasing number of production plants are certified in this system. In Norway there are two approved plants, there are three in Denmark and nine in Iceland, while there are more than 50 in Peru.

Marine Stewardship Council (MSC) is an independent, global, non-profit organization with certification and ecolabelling programs for fisheries and sustainable seafood (www.msc.org/). The MSC set science based standards, and the certification process is performed by an accredited third party in order to ensure independence. At present, there are 133 certified fisheries in the MSC program, among them a number of mackerel and herring fisheries in the North Atlantic, and 129 fisheries are under evaluation. A problem might be that different fisheries on the same stocks are certified independently. An example is mackerel fisheries, where several nations have fisheries and national quotas are set.

Friend of the Sea is a fisheries and aquaculture certification scheme promoted by the Earth Island Institute, an international not-for-profit and environmental organization. Friend of the Sea’s mission is to promote sustainable fisheries and aquaculture practices through the labeling and promoting of sustainable products on the markets. There are more than 50 certified fisheries worldwide.

Even though the marine ingredients are obtained from sustainable sources, and that the fisheries in question are being managed in compliance with the FAO Code of Conduct for Responsible Fishing, the global supply of fish meal and fish oil is no longer able to meet the increasing demand from an expanding aquaculture industry and, the aquaculture sector has done progress in research for alternative ingredients including plant products (Gatlin et al., 2007, Hardy 2010).

Keeping in mind the perspective of the regulatory framework on organic production, where the aim is a ”system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes” (EC 834/2007, rec. (1)), it is also important to evaluate to what extent each of the above mentioned aims is reached within the current regulations.

The environmental impact of providing feed for Atlantic salmon was assessed by LCA (Life Cycle Analyses), comparing certified organic to conventional feed (Pelletier and Tyedmers, 2007). Organic crop ingredients had lower life cycle impact compared to conventional crops, but this effect was totally outnumbered by the much larger impact of fish- or animal derived ingredients. Using fisheries by-products instead of fish meals and oils from reduction fisheries increases the environmental impact. The results of the study indicated that the current standards for organic salmon production with organic crop products and fish oil and meal from by-products failed to reduce the environmental impact of feed production as assessed in this study.

LCA was also used to compare three representative carnivorous finfish production systems: rainbow trout in freshwater raceways in France, sea bass in sea cages in Greece, and turbot (*Scophtalmus maximus*) in an inland recirculating system close to the seashore in France (Aubin et al., 2008). Results show that all the three systems contribute equally to the environmental eutrophication with their nitrogen and phosphorus emissions. While both sea bass and rainbow trout productions contribute to the climate change and acidification impact throughout feed production and net primary production use (NPPU), the turbot recirculating system contribute is accounted to its high energy consumption.

However, the limitations of the LCA methodology should be discussed, because there is no consensus on how the environmental impacts should be allocated between co-products in productions with multiple outputs (Ytrestøyl et al., 2011).

5. Conclusions and knowledge gaps

In line with the organic principles the animals' need for amino acids and fatty acids should be met primarily through natural feed compounds. Fish meal and fish oil are important components of this, particularly for carnivorous aquaculture animals, which have specific amino acid, fatty acid and other nutritional requirements, including minerals, vitamins and pigments.

However, using fish meal and fish oil only from trimmings, may negatively affect growth performance and environmental impact, and therefore conflict with contradict organic principles. Accordingly, to secure optimum performance, low environmental impact etc., it is recommended to consider that the diet for carnivorous fish might include fish meal derived not only from trimmings but also from whole fish, not used for human consumption and caught in sustainable fisheries until sufficient alternative sources of proteins and oils are available.

In conclusion, the following issues should be addressed to improve the current regulation in line with organic principles:

- Sourcing of feed ingredients for organic aquaculture need to be re-considered and supported by experimental data to secure compliance with the organic principles of fish welfare and environmental sustainability.
- At least until more knowledge is available fish meal and fish oil derived from industrial fish caught in sustainable fisheries, might be allowed as ingredients in feed for organic carnivorous fish. This includes feed for fry and brood-stock, as well as for on-growing fish, until sufficient alternative sources of protein and oil are available.
- The use of fish meal and phospholipids in the shrimps diet need to be re-considered.
- The use of other alternative feed ingredients providing high content of essential amino acids and lipids, where possible produced organically, might be used in priority to purified or free amino acids as feed supplements/additives.
- If not available from organic procedures, essential amino acids and lipids obtained by fermentation or other similar procedures might be considered.
- Studies have indicated that not only the overall dietary amino acid profile is important for efficient utilization of amino acids, but also the timing by which amino

acids from different protein sources appear in the blood stream after a meal. A significantly higher amount of indigestible carbohydrates have been measured in a diet based on vegetables than in a fish meal based diet, which suggested that the uptake of amino acids was affected by dietary carbohydrates. This issue also needs attention.

- Procedures in compliance with organic rules for removal of anti-nutrients in plant sources need to be addressed.
- Development of relevant organic plant sources to optimize the amino acid profile by mixing the protein sources and hence produce an optimum balanced diet for organic fish need to be considered.
- Important to keep focus on human health related to eating (organic) aquaculture products, including high content of omega-3 fatty acids (HUFAs) currently sourced from fish oil.
- Adjust regulation on request of exchanging fish oil by vegetable oils in accordance to development of vegetable or other sources producing omega-3 fatty acids (HUFAs).
- Research in alternative sources of omega-3 fatty acids (HUFAs) should be prioritized.
- Chemically well-defined analogic substances of minerals and vitamins may be considered for use if the natural substances are unavailable.

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Chapter 2: WELFARE, HEALTH, VETERINARY TREATMENTS AND BIOSECURITY

Chapter 2: Welfare, health, veterinary treatments and biosecurity 48 - 122

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1. Introduction, ethics and current regulation

1.1. Introduction

Among public and governments, there is increasing interest in the welfare of farmed fish. In addition, among farmers, there is growing awareness that good welfare equates to increased success of production activities (Lembo et al., 2010). Indeed, from a practical point of view, production efficiency, quality and quantity are often coupled with good welfare. As a result, fish welfare has become a growing area of research (Ashley 2007). Animal welfare is not easy to define. It is generally referred to as the physical and mental state of the animal interacting with its environment and associated variations (Chandroo et al., 2004). Most animal welfare definitions can be categorised into ‘function-based’, ‘nature-based’ or ‘feeling-based’.

a) ‘Function-based’ definitions basically assume that welfare is correlated with biological functioning, including physiological stress responses (Duncan 2005). This definition implies that if an animal is in good health and has proper functioning of bodily systems, it is experiencing good welfare.

b) ‘Nature-based definitions’ identify an animal to be in a state of good welfare if the animal “…is able to lead a natural life, expressing the same kinds of behaviour as it would in the wild, and is able to meet what are often called its ‘behavioural needs’…” (Huntingford and Kadri, 2008).

c) ‘Feeling-based’ definitions of welfare identify an animal to be in a state of good welfare if the animal “…is free of negative experiences such as pain, fear and hunger and has access to positive experiences, such as social companionship… (Huntingford and Kadri, 2008, 2009).” Welfare barely equals the current emotional state of the animal (Duncan and Dawkins, 1983) and, in the longer term, it represents the balance between positive and negative subjective experiences (Martins et al., 2012). The primary basis for the concept of animal welfare is the belief that animals are sentient, being capable to experience good or bad feelings or emotional states (Dawkins 1990).

Stress and stress-related responses should be considered as an adaptive condition of the organism that has the fundamental function of preserving the individual’s life. In addition, it is increasingly clear that individuality in stress reactions have to be included in the concept of animal welfare. Such differences often take the form of suites of traits, or stress coping styles (SCS), where traits like sympathetic reactivity, aggression and the tendency to follow and develop routines show positive relationships.

In aquaculture, fish are exposed to a range of industry practices that may act as chronic stressors which potentially compromise welfare. The effects of a wide range of aquaculture practices on the stress physiology of fish are well documented, and have been reviewed by Conte, (2004) and Pickering, (1992). Some of these practices include frequent handling, transport, periods of food deprivation, deteriorating water quality, sub-optimal stocking densities, fin-clips and social environments (Ashley 2007; Huntingford et al., 2006, Roques et al., 2010, Schram et al., 2010).

Determination of animal welfare requires the selection, collection and interpretation of different parameters and validated indicators. The Five Freedoms (see table 1) of animal welfare have become an accepted framework for evaluating suffering of farmed fish (FSBI, 2002 - Fish welfare; Lembo et al., 2010).

Table 1. five freedoms of animal welfare (FSBI, 2002)

Five Freedoms of animal welfare		**Indicators**
1	Freedom from hunger and thirst	Feed intake, growth rates, condition factor;
2	Freedom from discomfort	Physical damage: fin condition, cataracts, lesions, immune responses (e.g. lysozyme activity, respiratory burst activity, phagocytic activity);
3	Freedom from pain, injury or disease	Environmental monitoring: water quality monitoring (dissolved oxygen, ammonia, pH, carbon dioxide, suspended solids); Targeted sampling of fish: gill condition and checking for parasite infestation;
4	Freedom to express normal behaviour	Abnormal behaviour: swimming and feeding behaviour, distribution of the fish within a system (e.g. clumping around inflows), response of fish to an approaching farmer;
5	Freedom from fear and distress	Measuring primary and secondary stress responses: plasma, cortisol, glucose, lactate, muscles activity;

1.2. Ethics

Ethical consideration of fish can be founded in a number of reasons; respect for fish as a part of an ecosystem, focussing mainly on fish as species and its bioservices; respect for individual fish as fascinating living beings with an intrinsic value, having an individual life we could never have created; or (or in combination with) respecting individual fish as sentient beings, much in parallel to arguments for respecting dogs and pigs. Further, it is less usual, but of course theoretically possible, to act respectful towards individual fish out of repsect to humans, an argument often used in earlier days for preventing harm to farm animals (for the latter point, see Cserhalmi, 2004). Respect for fish species can however be based on such a line of argument, but is then rather related to considering the importance of fish for human livliehood. Sustainable fisheries benefit both fish species and humans, but the argument, then, is based on respect for the human need of fish for making their living (Bremer et al., 2012).

This is an ethically relevant point and much to the core in discussions not only on fisheries and quotas, but also on aquaculture. In organic aquaculture, a number of ethical issues are relevant to consider which are not linked to fish species or fish individuals. Some of the most important are (withoug ranking) fair labour conditions, economic viability in small and large scale production systems respectivley, relation between ’traditional’ and industrial production systems, environmental impact of production systems, as well as of feed and feed production systems and distribution, use of antibiotics, risk of escapees, loss of habitat for wild fish at farming sites, effects on immune system in wild population, and the general issue of whether or not to house carnivorous species. Some of these issues will be mentioned in this text, the focus however lies on issues related to the argument that concerns for fish are related to them being sentient creatures, i.e. welfare issues. Although widely debated for many years (Rose 2002), in today's debate on ethical concern for fish the most common foundation is related to fish as sentient beings. According to recent research in fish used for consumption the brain-behaviour relationships are not fundamentally different from those observed in mammals and fish are shown to adapt their behaviour to environmental changes, novel objects and react on pain and stressors. Hence, fish have capacities qualifying them to be considered as sentient beings. (Braithwaite et al., 2013). See also e.g. Braithwaite 2010, Chandroo 2004; Colin 2013 and EFSA 2009).

Relating ethics to the overall task of the project

Having a revision of EU regulation for organic aquaculture in mind, an important point of departure lies in the Lisbon Treaty (EC 2007) which came into force 2009 and states that *"In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals* (…) (Part one/Principles, Title II, Article 13). It is also stated in the EU directive 2010/63EU on animals used for research that all vertebrate animals are regarded sentient. Further, specific regulations on aquaculture EC Reg. 834/2007 (production conditions), EC Reg. 889/2008 (slaughter) and EC Reg. 710/2009 (transport) take as their point of departure, more or less explicit, that fish are sentient. Hence it is not a question of whether or not, but rather how to take this capacity into concern given there are other concerns to relate to and balance. Of further relevance for revision of organic regulation is the Article 11 in the Lisbon Treaty: *Environmental protection requirements must be integrated into the definition and implementation of the Union policies and activities, in particular with a view to promoting sustainable development*, as well as Article 12: *Consumer protection requirements shall be taken into account in defining and implementing other Union policies and activities*. These articles not only express a clear intention to ensure the values of sustainability and consumer protection are implemented into all future policies and establish a solid value basis for revision of organic regulation but are also de facto mirrored in the views expressed by some stakeholders (WP1).

Ethics and empirical studies

Before making use of the fact sheets and review papers, it is relevant to say a few words on the role of empirical studies in ethical thinking. In descriptive ethics, empirical studies are necessary to present and describe what is actually done in a certain context. Such studies are led by ethnologist, anthropologists or sociologists rather than ethicists, and make no claims on evaluating if certain behaviour is better or fairer than another. In normative ethics, on the other hand, empirical studies have another role. In this context, it is often said that the aim of all empirical ethics is to enhance context-sensitivity (Musschenga 2005) and to bridge the gap between empirical facts and normative statements (Molewijk et al., 2004). However, approaches differ on what is meant by context-sensitivity, what empirical research is thought to enhance, what is meant by ethical analysis, and how empirical research and ethical analysis are combined (Lund et al., 2006). In the present study the idea is to take results from empirical studies of fish into account for an ethical elaboration, i.e. for a discussion on ethically relevant issues. Combining empirical research with philosophical examination has the potential to give a more nuanced picture of important or difficult ethical issues than an ethical study based on pure theoretical theories, and in case a normative statement is requested, knowledge of praxis contributes to well founded decisions (Röcklinsberg 2001). Hence an ethical approach to empirical studies on fish might open for perspectives or questions not else raised. In the following a number of ethically relevant issues raised by organic aquaculture are mentioned and briefly discussed.

Consumers' perspectives

Given the structure of the project and the Treaty of Lisbon Article 11 and 12, sustainability and consumer protection requirements, as a point of departure, it is relevant to consider consumer perspectives. As presented in the round table discussions in Istanbul (the first Oraqua workshop with stakeholders), one of the main issues for consumers is related to their perception of organic production systems as closer to nature than conventional systems. This idea is well connected to a fundamental concept in IFOAM basic principles, naturalness, and calls for enabling fish in organic aquaculture to have a more natural life than fish in conventional farming. Consumers are also said to be ignorant about the differences between labels, which makes it plausible they don't really know what is required by a certain, e.g. organic, standard. The question then is whether the difference between organic and conventional aquaculture is large enough to gain consumer trust if informing better about the organic standards? If the actual production system is not evidently more natural, keeping consumer ignorance might seem to be a better choice, from a marketing perspective. This in turn leads to a need to handle a classical dilemma in organic standard setting – increase differences to conventional by stricter standards, taking the risk of loosing farmers/producers, or keep differences at a lover level, not necessarily minimum, but closer to conventional, in order to keep, or increase, number of certified producers, but at the risk of loosing consumers who dislike the 'weak' standards? Where is the break even with regard to the three parameters levels of standards, engaged producers, and consumer trust?

1.3. Current regulations

According to the current European legislation Reg. (EC) 834/2007, recital 1: "*Organic production is an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes. The organic production method thus plays a dual societal role, where it on the one hand provides for a specific market responding to a consumer demand for organic products, and on the other hand delivers public goods contributing to the protection of the environment and animal welfare, as well as to rural development*".

The organic production should meet animals' specie-specific behavioural needs. This concept is expressed repeatedly in the Commission Regulation (EC) 834/2007, in order to emphasize the different ways in which it is taken into account the fish welfare. Specifically, within the Reg. (EC) 834/2007 it is worth to mention:

Recital 17: "*Organic stock farming should respect high animal welfare standards and meet animals' species-specific behavioural needs while animal-health management should be based on disease prevention. In this respect, particular attention should be paid to housing conditions, husbandry practices and stocking densities. Moreover, the choice of breeds should take account of their capacity to adapt to local conditions. The implementing rules for livestock production and aquaculture production should at least ensure compliance with the provisions of the European Convention for the Protection of Animals kept for Farming purposes and the subsequent recommendations by its standing committee*".

Art. 3 (a)(iv) "*Organic production shall pursue the following general objectives: … (iv) respects high animal welfare standards and in particular meets animals' species-specific behavioural needs*".

Art. 15 1(b)(ii) "*husbandry practices, including feeding, design of installations, stocking densities and water quality shall ensure that the developmental, physiological and behavioural needs of animals are met.*"

The fish welfare during farming operations, such as transportation, as well as the minimization of any suffering of the animals, are also taken in consideration by the following articles:
Art. 15 1 (b)(v) "*transport shall ensure that the welfare of animals is maintained*".
Art. 15 1 (b)(vi) "*any suffering of the animals including the time of slaughtering shall be kept to a minimum*".

The Reg. (EC) 834/2007, besides fish welfare, gives great attention to the health of fish and disease prevention. To this purpose, it is worth to mention:
Art. 5 (e) "*Organic farming shall be based on the following specific principles: … (e) the maintenance of animal health by encouraging the natural immunological defence of the animal, as well as the selection of appropriate breeds and husbandry practices*".

Art. 15 1 (f)(i) "*disease prevention shall be based on keeping the animals in optimal conditions by appropriate siting, optimal design of the holdings, the application of good husbandry and management practices, including regular cleaning and disinfection of premises, high quality feed, appropriate stocking density, and breed and strain selection;*
(ii) disease shall be treated immediately to avoid suffering to the animal; chemically synthesised allopathic veterinary medicinal products including antibiotics may be used where necessary and under strict conditions, when the use of phytotherapeutic, homeopathic and other products is inappropriate. In particular restrictions with respect to courses of treatment and withdrawal periods shall be defined;
(iii) the use of immunological veterinary medicines is allowed;
(iv) treatments related to the protection of human and animal health imposed on the basis of Community legislation shall be allowed."

Art. 4 (a)(iii) "*Organic production shall be based on the following principles: … (a)(iii) exclude the use of GMOs and products produced from or by GMOs with the exception of veterinary medicinal products.*"

In addition, the Reg. (EC) 834/2007 promotes disease prevention by the biosecurity:

Art 4 (a)(iv) "*Organic production shall be based on the following principles: … (a)(iv) are based on risk assessment, and the use of precautionary and preventive measures, when appropriate*".

2. State of the art on husbandry – water quality

2.1. Current Regulation

According to Reg. EC 889/2008,

Article 6b (1) "*Operations shall be situated in locations that are not subject to contamination by products or substances not authorised for organic production, or pollutants that would compromise the organic nature of the products*".

Article 6b (3) "*An environmental assessment proportionate to the production unit shall be required for all new operations applying for organic production and producing more than 20 tonnes of aquaculture products per year*".

Article 6b (4) "*The operator shall provide a sustainable management plan proportionate to the production unit for aquaculture and seaweed harvesting. The plan shall be updated annually and shall detail the environmental effects of the operation, the environmental monitoring to be undertaken and list measures to be taken to minimise negative impacts …*".

Art. 25f (1): "*The husbandry environment of the aquaculture animals shall be designed in such a way that, in accordance with their species specific needs, the aquaculture animals shall: … (b) be kept in water of good quality with sufficient oxygen levels; (c) be kept in temperature and light conditions in accordance with the requirements of the species and having regard to the geographic location*".

Art. 25f (2): "*Stocking density is set out in Annex XIIIa by species or group of species. In considering the effects of stocking density on the welfare of farmed fish, the condition of the fish (such as fin damage, other injuries, growth rate, behaviour expressed and overall health) and the water quality shall be monitored*".

shall be monitored".

- *Annex XIIIa, for salmonids in fresh water: Ongrowing farm systems must be fed from open systems. The flow rate must ensure a minimum of 60% oxygen saturation for stock and must ensure their comfort and the elimination of farming effluent*
- *Maximum stocking density: salmon 20 kg* m^{-3}

Art. 25f (3) "*The design and construction of aquatic containment systems shall provide flow rates and physiochemical parameters that safeguard the animals' health and welfare and provide for their behavioural needs*".

Art. 25g: "*… 2. Rearing units on land shall meet the following conditions: (a) for flow-through systems it shall be possible to monitor and control the flow rate and water quality of both in-flowing and out-flowing water; … 3. Containment systems at sea shall: (a) be located where water flow, depth and water-body exchange rates are adequate to minimize the impact on the seabed and the surrounding water body; 4. Artificial heating or cooling of water shall*

only be permitted in hatcheries and nurseries. Natural borehole water may be used to heat or cool water at all stages of production.”.
Art. 25h *(3): “Aeration is permitted to ensure animal welfare and health, under the condition that mechanical aerators are preferably powered by renewable energy sources”;* (4): *“The use of oxygen is only permitted for uses linked to animal health requirements and critical periods of production or transport, in the following cases: (a) exceptional cases of temperature rise or drop in atmospheric pressure or accidental pollution*”, *b) occasional stock management procedures such as sampling and sorting, c) in order to ensure the survival of the farm stock.*

2.2. Current scientific knowledge

In aquaculture many kinds of fish and shellfish production systems, such as ponds, tanks, open and recycled systems, silos and cages are employed. All differ for the water characteristics in terms of quantity, quality, temperature, etc. Water is the medium in which farmed aquatic animal have to meet both their physiological and spatial needs. Deterioration of water quality can cause stress, reduce growth, increase the incidence of diseases to the point to be lethal for fish. Water quality is often referred to chemical parameters as concentration of dissolved oxygen, carbon dioxide, un-ionized ammonia-nitrogen, nitrite-nitrogen, alkalinity and calcium hardness (Conte 2004; Masser et al., 1999), as well as nitrate concentration, pH, alkalinity and chloride levels (Losordo et al., 1999).
There is a wide literature on the physiological and behavioural responses of fish to a wide variety of physical, chemical and biological stressors, including the typical ones common in aquaculture. In contrast, information on shellfish is highly limited (Sneddon et al., 2014).
The waste derived from fish feed and its metabolic end products, such as uneaten food, faeces and excreted, dissolved inorganic nutrients could seriously impair water quality. Recent studies have shown the efficiency of algae bio-filters in removing nitrogen from low ammonia fish effluents. Algae and zooplankton species developed in high-rate algae pond (HRAP) are commonly found in eutrophic water such as lagoons. For this reason, Deviller et al. (2004) tested the long-term effect of treated effluents on fish mortality and growth rates showing that the HRAP treated water, improving water quality through nitrogen reduction, prevent fish mortality, biological filter disturbance or delay in fish growth.
Attention has been drawn to oxygen and ammonia as the water quality parameters generating the observed density effects (Ellis et al., 2002). For example, increasing densities can reduce dissolved oxygen (DO) levels and increase un-ionised (UIA) concentrations in the water, depending on the pH (Ellis et al., 2002). As a result, low DO and high UIA levels, the latter being toxic to fish, can act as chronic stressors to rainbow trout, elevating plasma cortisol levels (Pickering et al., 1991).

Water quality in salmonids

The welfare in intensive salmon production is highly dependent on water quality; that in itself is dependent on several factors, such as oxygen, biomass, temperature and flow.

Oxygen

The demand of oxygen increases with increasing temperature. Dissolved oxygen is the first water quality variable that may limit production both in open and closed systems. Available oxygen is dependent on temperature and CO_2, but the usual recommendation for cold-water species is that they will have adequate oxygen as long as the dissolved oxygen does not fall below 80% (Wedemeyer, 1996). The threshold oxygen concentration for growth in rainbow trout has been shown to be ~75% saturation (Pedersen, 1987). For Atlantic salmon the optimal saturation of oxygen is 80 - 100%, but they can cope with 60% (Mattilsynet 2004).

Lack of oxygen (hypoxia) may be the major challenge in organic production, since the addition of oxygen is restricted through the regulations. In a study by Remen et al. (2014), they investigated performance in Atlantic salmon post-smolt exposed to cyclic hypoxia: 80% O_2 (control) or subjected to 1 h and 45 min of hypoxia (50, 60 or 70% O_2) every 6 h at 16 ^oC for 69 days. Cyclic hypoxia did not alter the oxygen uptake in fish, measured in night-time. Fish subjected to 50% and 60% O_2 reduced feeding by 13% and 6% compared with the controls, respectively, with corresponding reductions in specific growth rates.

Salmon parr exposed to 150% and 175% hyper oxygenated water produced higher levels of carbon dioxide with the subsequent decrease in water pH compared to control fish exposed to 100% O_2 (Espmark and Baeverfjord, 2009). At the 7th day of exposure the hyperoxic fish showed larger individual variation in swimming activity compared to the controls. The individual variance in activity, tail beat frequency and scattering in the tanks among super oxygenated fish decreased from the 7th to the 21st day of exposure. The behavioural effects of hyperoxia were seen in relation to altered feed consumption half way through the experiment, lower body weight, and altered haematological variables at day 21 of exposure. Plasma chloride was reduced in the exposed fish and haemoglobin decreased with increasing oxygen saturations. Plasma cortisol was elevated only in the 150% oxygenated group at day 21 (Espmark and Baeverfjord, 2009).

Espmark et al., (2010) investigated the development of gas bubbles caused by hyper oxygenation. The study was done to investigate the time course and exposure level associated with hyperoxia-induced gas bubble disease, through a combination of morphological and behavioural methods. Atlantic salmon pre-smolts were divided into three groups; one control group (no added oxygen), and two groups receiving gradually increasing oxygen saturations over three weeks, the high oxygen exposure group (130%, 160% and 220% O_2 saturation in week 1, 2 and 3, respectively) and the low oxygen exposure group (110%, 140% and 190% O_2 saturation in week 1, 2 and 3, respectively). The first signs of impaired water quality appeared 8 days after exposure start when a surface film of organic matter was observed in the high exposure group receiving 160% oxygen. The first signs of subcutaneous gas bubbles appeared after 14 days, in the high exposure group at a level of 160% oxygen saturation. After 16 days, 50% of the low oxygen group and 77% of the high oxygen group had gas bubbles on major parts of the body, at exposure levels of 190% and 220% respectively. The bubbles appeared on most of the fins, along the lateral line, on the gills, and in the eyes. Gas bubbles were detected by visual observations and the diagnosis was supported by histology.

Fish behaviour recordings revealed that during the first week, fish in the low oxygen group (110%) swam significantly more than both controls and high oxygen exposed fish. By week 3, at 190%, swimming activity was reduced. Fish exposed to 110% oxygen during week one also made more turns than the controls, and this difference persisted also in week 3 at 190%

exposure. Fish in both high and low oxygen exposed groups displayed more panic episodes compared to the controls, indicating physiological stress and possibly pain.

Temperature

Oxygen and temperature are tightly connected, and the demand for oxygen will increase as the temperature rises, since the oxygen is less available at higher temperatures. In an experiment by Hansen et al. (2015) that lasted for 51 days, salmon was subjected to an increase in temperature from 10^{o}C to 19^{o}C. Half-way through the experiment, from day 22, the fish were exposed to a reduction in oxygen saturation from 100% to 70%. The combination of higher temperature and lower oxygen saturation resulted in reduced growth and feed intake (Hansen et al., 2015).

Optimal rearing temperature in salmon production ranges between 8^{o}C and 14^{o}C (Marine Harvest, 2014). However, hyperthermic conditions, especially in the first stages of the salmon life may lead to spinal deformities. In a study by Ytteborg et al. (2010), they documented spinal deformities in fish that hatched at 10^{o}C and were exposed to 16^{o}C during first feeding, opposed to fish that hatched at 6^{o}C and were exposed to 10^{o}C at first feeding. In a series of publications Eriksen et al. (2006, 2007, 2008) showed that mild hyperthermia (10^{o}C) during incubation lead to better growth, but also different kinds of malformations (e.g. pelvic fin fluctuating asymmetry, bone deformities). The experiments also demonstrated the negative effects of prenatal stress, as some of the experimental fish were hatched from females that were exposed to cortisol just before egg stripping.

Biomass

We refer to the section of stocking density for review of effects of high densities per se. In this section, we will concentrate on water quality aspects from high biomass. Even though adding of oxygen is necessary to maintain sufficient water quality in intensive farming with high densities, adding of oxygen in excess may lead to increased water levels of CO_2 (> 20 mg L^{-1} (Smart 1981)) and reduced pH (Fivelstad and Binde, 1994; Espmark and Baeverfjord, 2009). Though oxygen is considered to be the primary limiting factor for fish performance, CO_2 and pH are considered as the secondary limiting factors (Fivelstad 2013). Long-term effect of hypercapnia is summarized by Fivelstad, (2013).

Flow

To ensure sufficient water quality in case no oxygen is added to the salmon tank, either the biomass or the velocity have to be adjusted. In the case of salmon smolt, the fish is standing against the current and swim with approximately the same speed as the water velocity (Espmark et al., 2015). Any deviation in velocity with the result that the fish either drift back-wards or move position much in the tank, is suggested to cause negative effects on welfare (Espmark et al. 2015). Thus, the velocity should not be increased to adjust for low oxygen levels. High water velocity may force the fish to exercise, thus improving health and welfare (Castro et al., 2011), however the higher swimming speed forced by fish due to velocity is also suggested to impair fin status (d'Orbcastel et al., 2009).

Water quality in sea bream and sea bass

Sea bream are born in the open sea during October-December. The species is very sensitive to low temperatures (lower lethal limit is 4°C). Juveniles typically migrate in early spring towards protected coastal waters, where they can find abundant trophic resources and milder temperatures. In late autumn sea bream returns to the open sea, where the adult fish breed (FAO 2005).

Although gilthead sea bream has been cultivated successfully for the last three decades in the Mediterranean area, cold waters in winter time affect fish growing performances in farms, even impairing fish health condition through the known "Winter Syndrome". The exposition to low temperature induces fasting, thermal stress, metabolic depression, alteration of the ionic equilibrium caused by malfunctioning of the gills and of the digestive system. Then fish becomes more susceptible to diseases for the impairment of the immune system (Ibarz et al., 2010).

Adult sea bass can withstand temperatures ranging from 2 to 32°C (Barnabé 1990), although Claireaux and Lagardère, (1999) quantified the temperature dependent metabolic performances. Indeed, between 10 and 25°C standard metabolic rate (SMR) increases from approximately 36 to 91 mg O_2 kg^{-1} h^{-1}. However, the increasing trend of the basal oxygen demand is not constant over all the temperature intervals, as between 20 and 25°C sea bass SMR is less susceptible to thermal fluctuations. Thus a temperature range 20-25°C corresponds to the thermal optimum of the species.

One of the most important aspects of the intensive aquaculture systems is the growth rate of fish. Many parameters contribute to modulate the growth rate. Garcia de la Serrana et al. (2012) showed that the most important non-genetic factor influencing growth rate is the temperature. The authors proved that the rearing temperature generates persistent effects on muscle growth patterns, with 20% more fibres of lower average diameter whether they are reared at lower temperature than optimal one, concluding that myogenesis and gene expression patterns during growth are not fixed, but can be modulated by temperature during the early stages of the life cycle.

Temperature is a limiting factor for fish growth through the effects on feeding and metabolism which can be also differently expressed according to the life stage. Evidences were reported by Barnabé, (1991) who observed that sea bass juveniles ceased growing at 11-15°C and grow fast at 22-25°C. Person-Le Ruyet et al. (2004) reported that total ammonia excretion is positively correlated to temperature as it is dependent on feeding rate. They also estimated that juvenile sea bass from a Mediterranean population has the maximum growth rate at 26°C, peaking within a narrow range of temperatures.

During the early life of cultured sea bream temperature has another important role on the development of anomalies and skeletal deformities such as folded gill-cover, haemal lordosis, deformities of the caudal and dorsal fin-supporting elements (Georgakopoulou et al., 2010). The prevalence of gill-cover deformities and caudal-fin deformities is higher when 16°C water temperature is used during the autotrophic and exotrophic larval periods. The exposition to 22°C resulted in the lowest and less variable incidence of haemal lordosis during the juvenile period and dorsal-fin deformities during the autotrophic and exotrophic phases.

Reproductive success is also influenced by environmental temperature. Motility rate and swimming velocity of spermatozoa decreases sharply 1.5 min after activation. After 2 hours the 20% of the spermatozoa remain motile, while exposition of spermatozoa to temperature under 10°C could decrease mobility parameters, although during the first 10 seconds after activation spermatozoa mobility is not impaired (Lahnsteiner and Caberlotto, 2012).

Temperature could also influence the typical management operation in aquaculture facilities, such as sedation and anaesthetization. Mylonas et al. (2005) demonstrated that lower temperature resulted in significantly longer anaesthesia induction and recovery time, presumably due to the positive relationship between temperature and opercular ventilation rates and metabolism. Indeed, for sea bream at 25°C the optimal concentrations of anaesthetics are determined to be 40 mg L-1 of clove oil, and 300 mg L^{-1} of 2-phenoxyethanol, while at a lower temperature (15°C) the optimal doses determined are higher (55 mg L^{-1} of clove oil and 450 mg L^{-1} of 2-phenoxyethanol) to reach both complete anaesthetization and recovery respectively in less than 3 and 10 min (Mylonas et al., 2005).

Acute temperature changes represent a realistic risk in aquaculture facilities where temperature may act as a stressor, particularly due to accentuated diurnal temperature cycles in shallow ponds or tanks, or due to accidental temperature shocks during water turnover. Under such conditions dissolved oxygen in intensive cultures may become an interacting limiting factor too.

It has also been shown (Person-Le Ruyet et al., 2009) that fin condition may be affected by metabolic activity under the control of ecological factors, such as temperature and O_2 concentration acting as limiting factors. In sea bass, fins were more eroded at elevated temperature than in cold water as fish are less active, especially when feeding: meal duration is shorter and daily feed intake less which, in turn, is responsible of lower growth rate (Person-Le Ruyet et al., 2004). This requires trade-off solutions for the aquaculture productions, because lowering temperature leads both to lower fin damages and to lower growth rates.

Gilthead sea bream is a euryhaline species, whose juvenile appears in low salinity lagoons and river estuaries. Juveniles reared in brackish waters showed the best growing performances at the salinity of 3.5 ppt, in which they increased their weight by 121%. Little variations of salinity slightly impaired sea bream growing performances (at 4.5 ppt weight increase was by 98%, at 2.5 ppt by 90%) (Appelbaum and Arockiaraj, 2009). This results show conclusively the possibility of utilizing inland brackish water for culturing gilthead sea bream as an additional alternative to traditional marine farming.

In sea bream exposed to different environmental salinity a "U-shaped" relationship was observed between environmental salinity and gill Na^+/K^+-ATPase activity, in both long and short-term exposure, with the increase in activity occurring between 24 and 96 hours after the onset of exposure (Laiz-Carrión et al., 2005). Moreover, plasma osmolality and plasma ions (sodium, chloride, calcium and potassium) showed a tendency to increase in parallel with salinity.

The stressful effect that environmental salinity has in acclimation of gilthead sea bream is also reflected by the relative modulation of the immune system, acclimation to a wide range of salinities from low saline water (LSW, 6 ppt) to hypersaline water (HSW, 55 ppt) alters the humoral immune response. In general, hypo-osmotic acclimation has a negative effect, while hyper-osmotic acclimation has a beneficial effect on sea bream humoral immune parameters. These results might be explained by the effects of osmoregulatory hormones and the involvement of different organs in the immune and osmoregulatory responses (Cuesta et al., 2005).

Sea bass is also a euryhaline marine fish. The sea bass capabilities to adapt and live in this wide range of salinity are shown by fish under acclimated or adapted conditions. Acute salinity changes induced a transient increase in the metabolic rate of the fish (Dalla Via et al., 1998). The authors demonstrated that the stepwise (increasing and decreasing) changes of external salinity produce metabolic rates increasing at each salinity change, up to 80% of the metabolic rate. The rate could remain elevated up to 10 hours after the salinity change, aggravating the dissolved oxygen conditions in the tank or pond, thus further reducing the maximum sustainable stocking density (Dalla Via et al., 1998). Sea bass juveniles have a low saline preferendum, indeed they are known to frequent estuaries and lagoons where salinity is lower than in the open sea. Saillant et al. (2003b) demonstrated that sex determination is not directly modulated by the salinity level but seems to be subjected to complex environmental regulations.

Dissolved oxygen concentration is surely among the most important environmental variable for all fish species, as well as for sea bream. Sea bream is more sensitive to hypoxia than sea bass. Oxygen concentration of 5 mg L^{-1} is the minimum required by sea bream during grow-out (Okte 2002).

Dissolved oxygen is often low in polluted waters and many of the physiological responses of fish to chemical pollutants, at acute concentrations, are similar to those produced in response to environmental hypoxia (Heath 1995). Hypoxia is not limited to freshwater habitats. Indeed, oxygen levels in the oceans vary with the depth, temperature, salinity and productivity (Bushnell et al., 1990).

The concentration of oxygen available to fish varies across different production systems. In cages, dissolved oxygen can be a limiting factor at high summer temperatures. Such problems do not normally arise in flow-through or recirculating systems except in the event of mechanical breakdown. At 40% oxygen saturation, feed intake and growth are impaired in sea bream (EFSA 2008).

The environmental oxygen availability could generate physiological as well as morphological modification in sea bass specimens. Saroglia et al. (2002) reported that the total respiratory surface area (RSA) of sea bass exposed to different oxygen partial pressures (respectively 60, 90 and 140% saturation) corresponded negatively with oxygen availability in the water. In the same experiment, no significant differences in the total length of filaments or frequency of lamellae were observed, although the total length of filaments was shorter in fish cultured under hyperoxia.

Pure oxygen is largely utilised in modern land-based aquaculture in order to rear fish at high densities, with significant physiological advantage. Experimental data (Saroglia et al., 2000) showed that both environmental temperature and dissolved oxygen concentration affected the blood-to-water diffusion barrier, known as Gas Diffusion Distance (GDD). GDD increased with the increasing of dissolved oxygen (DO), both due to reduced water temperature and to the mild oxygen hypersaturation following application of pure oxygen. The advantage for fish may be found in the compromise between maximising O_2 diffusion at the gills and ions/water intake/loss, known as “osmoregulatory compromise”.

In intensive fish culture systems, a reduced availability of dissolved oxygen in water is often observed. This is ascribed to a high fish density and to the feeding practices; algal blooms and elevated temperatures can contribute as well. This lack of oxygen can induce responses and the typical metabolic adjustments caused by the hypoxic stress are activated to maintain oxygen supplies in the critical organs and to reduce consumption of oxygen. Some adaptive mechanisms can change fish gene expression with the aim of saving oxygen. Indeed, in sea bream changes in haemoglobin patterns have been reported due to hypoxia conditions, even if the two major components of sea bream haemoglobin are functionally very similar (Campo et al., 2008).

Oxygen concentration in water is a pivotal factor that contributes to modulate fish sensibility to other water quality parameters. It was demonstrated, for example, that juveniles of sea bream exhibit increased sensitivity to ammonia in case of oxygen saturation drops below 85% of saturation, while increased mortality occurs when the saturation is below 40% (Wajsbrot et al., 1991).

Sea bass basal oxygen demand is not constant. In cold conditions (10-15°C) metabolic processes are strongly temperature-dependent, while between 20 and 25°C fish became less susceptible to thermal fluctuations. The range of the standard metabolic rate (SMR) increases from approximately 36 to 91 mg O_2 kg^{-1} h^{-1}, is between 10 and 25°C. Indeed, also the significant interaction between temperature and oxygen concentration suggests that the higher is the temperature, the greater is the energetic expenditure (Claireaux and Lagardère, 1999).

Dalla Via et al. (1989) proposed a mathematical model to assess the maximum stocking density in function of oxygen supply and temperature. Only an increase in oxygen supply allows higher stocking densities. Oxygen supply by air bubbling is not always the appropriate approach, since early stages of sea bass are highly affected by water currents and turbulences. An increase in the water exchange rate with well-oxygenated water is often chosen for this purpose.

Water quality in common carp and carp pond species

The appropriate pond water quality is one of the principal prerequisites for proper pond management. This means maintenance of carefully balanced fish stock, sufficient manuring/fertilization and rational supplementary feeding in accordance to the size of the standing stock of fish. As production intensifies, the improvement of water quality has to be ensured by aeration and/or the exchange of water. The calculation of production in ponds is based on the unit area, such as the number of fish per unit area (fish/hectare) and/or biomass of fish per unit area (kg/hectare, or t/hectare) (Woynarovich et al., 2010). The important physical characteristics of pond water with direct effects on fish production and welfare issues are:

Temperature which determines the growth, production and reproductive activity of all aquatic organisms. When the water temperature is extremely low, fish stop feeding and remain dormant at the pond bottom. Extremely high temperature in carp ponds is, contrarily associated with increased fish metabolism and decreased concentration of dissolved oxygen, which often leads to suffocation and gasping.

During its domestication the common carp has adopted to European climate conditions. Production ponds provide temperature conditions almost identical to natural. The annual temperature cycle determines the temperature in ponds and thus carp activity. Carp are able to survive across a wide temperature range (from ~0°C to > 36°C), although activity changes drastically with temperature. Lethal temperature for small individuals is slightly higher than for adults (respectively ~38°C for small fish and ~36°C for adults). Such high temperatures are very unusual in normal production conditions in Europe. Optimum feeding efficiency and growth rate is generally obtained between 23-29°C. Decreases in water temperature below 20°C and increases above 30°C both lead to a decline in feeding. Carp will increase weight when feeding at temperatures above ~14°C. This means that in most European carp producing countries, there is a growth period of about 150 to 160 days during the year.

Temperature requirements of carp depend substantially upon the life stage: (1) egg and free embryos and swimming larvae in hatcheries, (2) fry for pond stocking and (3) on-growers in production ponds. Temperature shocks usually do not occur during the production cycle but they may happen quite frequently during fish handling and transportation.

In carp pond polyculture, the production season begins when water temperature is constantly over 10°C. Apart from pike and pikeperch, the majority of species start intensive feeding above 15 – 20°C. The mentioned predators feed intensively in colder water, while the appetite of Chinese major carps increases when the water temperature is over 20°C.

Since the optimal range of water temperature required for the intensive growth of carps is between 20 – 25°C, the rearing period begins in spring and ends in autumn. In this period, the daily average water temperature permanently remains near to or over 20°C. This period is called production (or growing) season. Its actual length depends on the number of warm months, which varies according to geographical regions and altitude. Due to more or less stable outdoor conditions, the temperature shocks usually do not occur during the production cycle.

Density or **specific weight** of water changes with the temperature. The specific weight of warm water is lower than that of cooler water. This physical characteristic is the reason why the calm or undisturbed water stratifies in layers. The cold water sinks down, while water of higher temperature stratifies on the surface. As a consequence, a diurnal vertical circulation can develop in ponds during the days when the wind does not create other currents. This process means that the upper layer of water contacting with relatively cold air at night cools down more quickly than the water at the pond bottom. The specific weight of this cooler water increases. Therefore, the surface water sinks to the bottom and the warmer and lighter water from the bottom rises to the surface. This circulation can transfer oxygen to the bottom but also ensures the exchange of nutrients between water and mud. Because of the chemical composition and molecular form of water, when water cools down in winter the specific weight increases only up to 4°C. At this value the specific weight is the highest, which is 1 g ml^{-1}. At lower temperatures than 4°C, the specific weight of water again decreases. This is the reason why ice floats on the surface. Consequently, under the ice, in deep, undisturbed waterbodies, the water temperature is always 4°C at the bottom. If the water is deep enough, this phenomenon protects the fish from freezing and enables their successful overwintering.

Movement of pond water also has an important effect on the pond ecosystem. Wind, thermal circulation of water and currents developed by inflowing and outflowing water create horizontal and vertical streams in ponds. These movements ensure healthy pond life through supporting the exchange of gases and dissolving nutrients to and from the pond bottom.

Carp do not require flowing water. Their natural habitat is slow flowing or standing waters. In farming conditions carp are produced in ponds without water flow, except for replacement of water losses due to seepage or evaporation.

The **pH** value is an important parameter of pond water. Fish farmers should know and regularly check the pH of pond water, because the pond fish welfare and all chemical and biological processes which determine the production depend on this. Among others, pH influences the solubility of and accessibility to the different minerals.

In fish ponds where the density of phytoplankton is high, the daily fluctuation of pH is considerable. This is because in the course of assimilation (photosynthesis) and dissimilation (respiration) phytoplankton reduces or increases the concentration of carbon dioxide (CO_2). During daytime, when phytoplankton assimilates, it consumes CO_2 therefore pH increases. At night, when the plants dissimilate, they consume oxygen and produce CO_2. This decreases the pH of the water.

Water ranging in pH between 6.5 and 9.0 before dawn is considered the most suitable for pond fish culture. At pH 6.5 – 5.5, fish production will be less, either because of the direct effect on the fish and/or on the growth of fish food organisms. Acid water with pH 5.0 – 5.5 can be harmful to fish. On the ther hand, water with excessive alkalinity (above 10) can also be harmful to fish (Hepher and Pruginin, 1981). Adult carp can survive in waters with a wide range of pH 5.5 – 10, although extreme values reduce feeding activity and thus growth rates. Optimum pH values are between 7 and 8. In production ponds pH rarely drops to below 6, though it may often exceed pH 9 (reaching pHs of up to 11) during spring algal blooms. The effect is greater in fry ponds with low water turbidity. The direct effect of pH is less important than the indirect effect of increased pH values have on the un-ionized ammonia content.

The current situation in a large number of carp pond ecosystems is characterized by a high biomass of phytoplankton. Such situation often occurs already in early spring and usually culminates in May. This is the period when phytoplankton can induce increased pH values with its photosynthetic activity even above 10. It is caused by a sufficient amount of nutrients enabling fast growth of phytoplankton biomass, light conditions in spring (high solar radiation, length of days) and lower respiration of the entire planktonic community and sediments. While photosynthesis is not significantly slowed down at lower temperatures, respiration is substantially dependent on temperature and when the temperature is lower, respiration is also markedly lower. Alkalinization occurs during the photosynthesis process because carbon dioxide is being used up, and pH values thus increase due to the decrease in carbonic acid. This process is also characterized by fluctuation of pH values within the range of approximately 1 degree during a day. Breathing releases carbon dioxide and it thus causes acidification. Therefore, predominance of photosynthetic processes in spring induces a rapid growth in pH values in this period. Another high development of phytoplankton occurs in summer when temperature exceeds approximately 16°C which, however, results in increased respiration of both plankton and sediments. It is possible to notice a growth in available nutrients, ammonia and phosphates at the beginning of summer that are intensively released from sediments or possibly applied fertilizers. It naturally stimulates development of phytoplankton, but at the same time the intensity of respiration processes rises as well. Paradoxically, it results in the decrease in pH values despite the fact that phytoplankton biomass may be even larger than in spring.

Naturally, extreme pH values of pond water influence fish welfare. Low pH (< 5) values, which may damage sensitive fish tissues, occur mainly in early spring period due to the snow melting. On the other hand, high pH values (late spring) are dangerous due to their impact upon the release of toxic free ammonia from non-toxic ammonium ions resulting in serious damages of fish gill apparatus by „self-intoxication“ and subsequent branchionecrosis and branchiomycosis leading often to heavy losses (see the paragraph „Oxygen“ below).

Many gases and solid materials dissolve well in pond water, which is explained by the molecular structure of water. Gases dissolved in water derive either from the air, from the pond bottom, or they are produced during the metabolism of different living organisms. Oxygen, carbon dioxide, sulphur hydrogen, free ammonia and methane are the gases which can have both supportive and harmful effects on the aquatic life in general and on fish in particular.

Oxygen (O_2) dissolves well in water. Dissolved oxygen in pond water ensures the respiration of fish. The amount of dissolved oxygen in the water increases as atmospheric pressure rises, but decreases when the temperature rises. These physical effects have very practical consequences. When water temperature increases, fish metabolic rate increases at just the same time as oxygen availability is limited. In production ponds available oxygen is also consumed by microorganisms that decompose organic matter in bottom sediments, which limits the availability of oxygen for fish.

Oxygen can penetrate into the water from the atmosphere, but the majority of DO in pond water is produced by phytoplankton in the course of photosynthesis. Because of intensive photosynthesis, water can be temporarily oversaturated by oxygen. The oxygen in excess is either consumed or it disappears into the atmosphere.

Deterioration of light conditions caused by “self-shading” by dense phytoplankton plays an important role in functioning of the pond ecosystem with respect to its oxygen regime. The end of August and the beginning of September usually represent a critical period when the decrease in oxygen concentration up to values critical for fish stock survival can occur during the night as a result of intensive respiration of plankton and sediments at continuing higher temperatures and decreased intensity of photosynthesis due to marked shortening of a light period (Pechar et al., 2002). Such deficit deepens in particular mainly in early morning hours (in August and September, it is between 4 to 6 o’clock in the morning) as the “start-up” of photosynthesis is approximately one to two hours delayed behind the light. From the vertical gradient point of view, despite shallow depth of ponds, significant oversaturation of surface layers with oxygen during light parts of sunny days due to intensive assimilative phytoplankton activity is apparent when the trophic level is higher. On the contrary, there is usually a lack of oxygen at the bottom because there is not enough light and the increased content of organic matter in mud is subject to bacterial decomposition which is associated with continuous oxygen depletion (“sediment respiration”).

Water can dissolve only a certain quantity of oxygen at a certain temperature. The possible maximum oxygen content of water (100% saturation) depends on the actual water temperature and the partial pressure of oxygen in the atmosphere. Dissolved oxygen content changes slightly with the quality and quantity of dissolved materials. The altitude also modifies the oxygen content of the water.

In general, it can be stated that an enormous increase in phytoplankton biomass has occurred in many carp ponds (basically, in all lentic waters, mainly valley reservoirs) in the last decades. The biomass has caused, in consequence of high biological activity, destabilization of their ecosystems and it has been connected with a considerable fluctuation of key parameters of the aquatic environment (concentration of oxygen and free ammonia, pH values). It has thus largely increased the probability of occurrence of situations when some parameters exceed critical (threshold) values often with fatal consequences for the pond ecosystem. These fluctuations represent a natural reaction to high and unbalanced nutrient loading and behaviour of the entire ecosystem becomes hard to predict.

Significant negative changes in a pond ecosystem occur also in connection with an excessive development of large daphnia dominated zooplankton. This status can be usually observed in hypertrophic ponds with point pollution sources (wastewater discharge rich in nutrients and easily decomposable organic substances) and then in ponds with diffuse pollution sources (nutrient wash, mainly of nitrates and phosphates from surrounding land). Phytoplankton develops in these hypertrophic and usually largely saprobic ponds in spring and due to its intensive photosynthetic assimilation carbon dioxide is withdrawn from the carbonate system in water. This phenomenon decreases the neutralization capacity of water. As a result of that, water pH exceeds values higher than 10. High concentrations of total amonium ions (NH_4^+ + NH_3) tend to occur in these ponds too which leads to high concentrations of free (non-dissociated – toxic to fish) ammonia (NH_3) due to high pH values. At this period, there is usually a sufficient amount of oxygen in water (usually oversaturation caused by photosynthetic assimilation) and that is why fish tolerate the above-mentioned unfavourable conditions for a certain period. In a subsequent period that is usually accompanied by increasing water temperature, rapid development of filtering zooplankton occurs in these ponds as well. The filtering zooplankton, in addition to its own high oxygen consumption, reduces heavily the oxygen producers (phytoplankton) due to its filtering pressure and, what is more, it also releases ammonia as its metabolic product. Reduction of phytoplankton manifests itself with a decrease in the chlorophyll concentration in water and with increased water transparency. Next, as a result of phytoplankton decrease, the concentration of oxygen dissolved in water sharply decreases (within one to two days it changes from oversaturation to deficit concentrations). It is exactly the decrease in oxygen content in water that represents a critical moment for an outbreak of toxic gill necrosis usually connected with damage (see the paragraph „pH" above) or even death of a large part of a fish stock (even a minor damage of the branchial apparatus of fish causes a heavy decrease in grazing pressure of a fish stock, which further supports development of large daphnia dominated zooplankton and intensifies even more the oxygen deficit). At the same time, water pH value decreases as well, however, at the beginning, the decrease is very slow.

Carp are resistant to low oxygen concentration as an evolutionary adaptation to environmental conditions that occur in their natural habitat. At oxygen concentrations below ~2-3 mg O_2 L^{-1} (~20 - 30% saturation) carp feeding activity and hence growth rate decrease. For the first few months of the life cycle, fry are sensitive to low oxygen concentrations. Dissolved oxygen at concentrations above 3-4 mg O_2 L^{-1} does not affect carp performance.

The most production technique for common carp (earth ponds, at less than 1500 kg ha-^{1}) usually provides adequate oxygen at normal stocking levels without recourse to aeration Accidental oxygen depletion, occurring in August/September (see above), can affect fish health or cause mortality by direct asphyxia or lowering resistance to secondary bacterial infections. Such low oxygen levels usually only last for a few days.

Free carbon dioxide (CO_2) is important for the photosynthesis of green plants because it is the source of carbon, which is one of the main components of all organic materials. A small quantity of CO_2 may penetrate from the atmosphere into the water. However, the majority of carbon dioxide is the result of the respiration of living organisms.

Ammonia is produced by different groups of living organisms as the end product of their metabolism. In the course of respiration, fish excrete through the gills about one-third of the consumed nitrogen in the form of ammonia. Free ammonia (NH_3) and ammonium ion (NH_4^+) together represent the total ammonia ($NH_3 + NH_4^+$) content of pond water (see paragraphs „pH“ and „Oxygen“ above).

Hydrogen sulphide (H_2S) is produced by anaerobic bacterial decomposition of proteins, degraded organic materials and sulphates in the mud of the pond bottom. Sulphur hydrogen dissolves very well in water. It is a strong fish poison especially when the pH of the water is acidic.

Dissolved salts characterize the natural waters as they always contain some of the eight macro-ions of sodium (Na^+), potassium (K^+), calcium (Ca^{++}), magnesium (Mg^{++}), carbonate (CO_3^-), hydrogen-carbonate (CO_3^-), chlorine (Cl^-) and sulphate (SO_4^-). The total concentration of salts is also an important parameter of waters. The salt concentration is expressed by weight (mg l^{-1}) or by percentage or is described by the electric conductivity of the water. Different dissolved nitrogen forms, phosphorus and organic materials are also found in pond waters. All of the dissolved materials have an outstanding role in fish ponds because they can be either micronutrients and direct or indirect food sources of the aquatic organisms.

Suspended solids in increased concentrations are a typical feature of carp ponds. Feeding activity of carp causes re-suspension of bottom sediments, resulting in high suspended solids concentration (Adámek and Maršálek, 2013). Carp can re-suspend 4 - 6 times more bottom soils than their own fish biomass a day (Breukelaar et al., 1994). Type of suspended matter depends on original soil composition, age of pond and feeding rate (type). Usually more than 95% of the re-suspended material consists of mineral particles, usually fine clay particles. Carp in production ponds are exposed to suspended solid concentrations of up to ~200 mg L^{-1}. Suspended solid concentrations caused by feeding behaviour under most common production conditions do not affect fish health status. In contrast, clear water in a carp pond is a sign of possible problems like fish kill, extremely high biomass of filtering zooplankton, reduced food consumption of the pond stock and others.

Increased concentration of suspended solids may cause serious problems also in hatcheries. Their increased sedimentation in incubation jars and troughs may result in heavy losses or even total kill of developing eggs and/or hatched free embryos.

2.3. Conclusion and research gaps

-Fish and shellfish are poikilothermic animals, which means that temperature is the main factor playing on their metabolism and quick temperature shifts do impact directly and indirectly their welfare;

-Water quality parameters are species-specific, but the first limiting factor is often oxygen;

-Fish farms should be located where the water quality, and hydrodynamics ensure compliance with animal welfare.

Specific for salmon, in Annex XIIIa: Oxygen levels should be maintained at 80% instead of 60%. With the numerous of documented advantages with the use of oxygen in intensive farming, we advise that the restrictions of using oxygen is loosened up. We advise not to use velocity to control the oxygen. The maximal density without oxygen without negative welfare effects is a knowledge gap.

3. State of the art on husbandry - Light and photoperiod

3.1. Current regulation

According to Reg. EC 889/2008,

Art. 25f (1): "*The husbandry environment of the aquaculture animals shall be designed in such a way that, in accordance with their species specific needs, the aquaculture animals shall: … (c) be kept in temperature and light conditions in accordance with the requirements of the species and having regard to the geographic location*".

Art. 25h (2): "*The following restrictions shall apply to the use of artificial light: (a) for prolonging natural day-length it shall not exceed a maximum that respects the ethological needs, geographical conditions and general health of farmed animals, this maximum shall not exceed 16 hours per day, except for reproductive purposes; (b) Abrupt changes in light intensity shall be avoided at the changeover time by the use of dimmable lights or background lighting*".

Art. 25s (5): "*Ultraviolet light and ozone may be used only in hatcheries and nurseries*".

3.2. Current scientific knowledge

Light regime requirements for sea bream and sea bass

Light represents an important environmental factor that could influence severely fish behaviour and physiology. There is are also evidence of photoperiod influences on changes in the humoral innate immune system in sea bass as well as in sea bream. Esteban et al. (2006) described a clear circadian rhythm of the immune parameters, which may be related to the melatonin levels present at each time during the day. Moreover, complement activity reaches the highest value during light hours and the lowest during dark hours, while peroxidase activity follows just the opposite pattern, decreasing during light hours and increasing during dark hours, in both sea bream and sea bass. Lysozyme activity also follows a clear daily rhythm, although a different inter-specific pattern was observed between sea bream and sea bass.

There is evidence of the existence of circadian systems involving light as synchronizer of the rhythm or modulating feeding behaviour. Cedrá-Reverter et al. (1998), during studies on plasma glucose, insulin, and cortisol in sea bass held under different photoperiod regimes showed that plasma glucose rhythm is controlled by the photoperiod. Moreover, it was observed by Del Pozo et al. (2013) that sea bass blood glucose shows daily variations during both winter and spring seasons. This physiological aspect coincides with their change in feeding rhythms from diurnal to nocturnal in winter, returning to diurnal feeders in spring.

Also the capacity of digestion in sea bream is proven to be somewhat influenced by the light cycles at diurnal and annual level. Indeed, the acid protease activity shows a rhythm with a 6-months period (bimodal rhythm) with acrophases in May and November, while activities of pancreatic enzymes exhibited parallel changes with two peaks of activity in January and October without a specific rhythmic pattern. Moreover, the daily changes in enzyme activities are significant only in May, June, and November for basic proteases and May, October, and November for amylase (Sánchez-Muros et al., 2013).

There is also evidence of photoperiod influence on changes in the humoral innate immune system in sea bass as well as in sea bream. Esteban et al. (2006) described a clear circadian rhythm of the immune parameters, which may be related with the melatonin levels present at each time during the day. Moreover, complement activity reaches the highest value during light hours and the lowest during dark hours, while peroxidase activity follows just the opposite pattern, decreasing during light hours and increasing during dark hours, in both fish species. Lysozyme activity also follows a clear daily rhythm, although a different inter-specific pattern was observed.

Sea bream begins gonadal development during September in preparation for winter spawning which starts around late December to early January in the eastern Mediterranean region, while the onset occurs earlier in the western part of the basin. Kissil et al. (2001) used successfully photoperiod manipulation to hasten growth (Ginés et al., 2004), development and survival of young stages of gilthead sea bream. In addition, modified photoperiod regimes have also been successfully used to alter the rate of sexual maturation and time of spawning of sea bream (Zohar et al., 1995). It was also observed that skin luminosity was directly related to the number of hours to light exposure (Ginés et al., 2004). Moreover, the postponement of gonadal development resulted particularly useful in aquaculture since somatic growth continues because energy sources from the feed, normally deployed in gonad maturation are shunted into somatic growth with the resulting added weight gain.

Zanuy et al. (2001) reviewed the latest scientific advances on the genetic and physiological control of the sex and the process of puberty. The author reported that photoperiod is actually considered as one of the most important environmental parameter triggering puberty and reproduction in fish, well demonstrated in marine fish species, including the sea bass (Bromage et al., 1993). Indeed, sea bass exposed for three consecutive years to artificial photoperiods (LO: constant 15L:9D; EX: extended natural photoperiod along 18 month cycle), during the first reproductive season had significantly lower GSI than control group. The latter group, similarly, showed a significantly higher proportion of spermiating males, inversely correlated with somatic growth. Alterations of environmental factors, such as the photoperiod, influence the timing of the onset of puberty in male sea bass, inducing changes of activity of the brain–pituitary–gonad (BPG) axis. Although more research is needed to clarify the underlying mechanisms controlling puberty in male sea bass, it seems that sea bass Gonadotropin-Release Hormone (sbGnRH) is the only form involved in the onset of puberty and that, apparently, pituitary gonadotropin GtH II (LH) has an important role in the testicular maturation.

Also the quality of light, as well as the photoperiodic regime, has proved to be effective for sea bream welfare. Exposition of specimens of this species to red light (605 nm) increases brain dopaminergic activity, while a tendency towards reduced growth is also observed (Karakatsouli et al., 2007). On the other hand , results obtained for sea bream related to the remaining light colours (blue and white) were not clear enough to permit a definite choice of a specific light colour for a more efficient farming of sea bream (Karakatsouli et al., 2007).

Light regime requirements for Atlantic salmon

Atlantic salmon parr and smolt are generally reared in deep, indoor tanks using high energy artificial lights. Cage held salmon can also be subject to artificial lighting in the seawater phase. Although photoperiod manipulation, in the form of manipulating absolute daylength, can be used in production regimes to control smoltification and produce out-of-season smolts (e.g. Berrill et al., 2003), or control reproduction (e.g. Bromage et al., 2001), the impacts of these and other parameters upon fish welfare are rarely considered. EFSA (2008) have stated “The welfare consequences of artificial photoperiod treatments are not fully known. Extensive use in industry has not so far revealed any negative welfare effects of use of continuous light, and the use of light is an efficient way of reducing the welfare problems associated with salmon maturing in sea-water.”

Abrupt changes in the shift from the dark cycle to the light cycle (and vice versa) have been shown to induce a rapid downwards swimming behavioural response in Atlantic salmon in tanks (Mork and Gulbrandsen, 1994). The authors stated “A rapid change in activity is interpreted as a stress response and it is concluded that sudden transitions between darkness and light, no matter how small, should be avoided in fish farming.” Folkedal et al. (2010) looked at whether parr could habituate to this sudden transition. They used 9 tanks of ca 120 g fish, 585 fish per tank at densities of 15.2 kg m^{-3} and studied the oxygen consumption at the tank level. Fish can habituate to this sudden transition and authors stated “The results show that parr effectively habituated to the strong stimulus, but that 31% of the initial response was sustained over weeks and not subject to habituation”.

There are some potential welfare benefits to reducing the incidence of maturation in farmed salmon, such as reduced feeding and elevated risks and sensitivity to disease (see Bromage et al., 2001). However, the imposition of artificial lighting regimes in both freshwater and seawater needs evaluated to identify and further potential welfare benefits or costs of the strategy.

Very little work has been done on the welfare implications of continuous lighting upon salmon welfare in freshwater out-of-season smolt production, aside from statements suggesting synchronised smolting and better quality smolts reduces mortality after seawater transfer (EFSA, 2008 and references therein)

Fjelldal et al. (2005) looked at Atlantic salmon vertebrae in relation to photoperiod in 75 g post-smolt Atlantic salmon. They found that fish reared under continuous light grew better but had significantly lower mineral content and vertebrae mechanical strength than fish reared under a natural photoperiod. Fjelldal et al. (2012a) looked at the effect of continuous light on vertebral morphology in relation to diet in 230 g post-smolt. They found continuous light with sufficient dietary phosphorus levels did not result in bone deformities or lower mineral content. In addition, Wargelius et al. (2009) looked at the effects of continuous light on vertebral mineralization and deformities of post-smolts. They reported that continuous light affected vertebral mineralization, but this did not lead to any difference in vertebral deformities between continuous and natural light regimes. Moreover, Migaud et al., (2007) looked at the effects of difference light sources, spectral composition and light intensity on cortisol levels and retinal damage in Atlantic salmon post-smolts. In the study, the authors compared white LED, and low and high intensity blue LED on ca. 800 g tank held post-smolts. It was observed that high intensity blue LED light increased plasma cortisol and glucose levels within 3 h, returning to a basal state 24 h post-light onset. This was not observed at lower blue LED light intensities and fish under white LED lighting. No retinal damage was observed in any treatment. Oppedal et al., (2003) looked at the effects of continuous light regimes versus simulated natural photoperiod in tank-held salmon post-smolts, and noted an appetite depression in the continuous light groups for the first 6 – 8 weeks after the fish were subject to 24h light.

The use of artificial light in cage-held adult salmon can be used to direct swimming behaviour in terms of both depth and density (Juell et al., 2003). Further, Frenzl et al., (2014) reported that submerged lighting at 10 m depth in sea cages reduced the number of sea lice on fish in comparison to fish subject to lighting at 1.5 m deep.

Light requirements for common carp and carp pond species

Light conditions in pond water correspond to natural photoperiod and determine the intensity of the photosynthesis. Light conditions in a pond depend on the transparency of water, which is influenced by turbidity, water colour and by biological factors such as density of plankton and the number and size of the different fish species. Transparent water allows an intense penetration of sun, which is important for primary producers - phytoplankton and macrophytes. The intense penetration of light supports the intensive growth of macrovegetation, which is less desirable in fish ponds.

Carp, like virtually all fish, reacts to light changes. Thus, change in photoperiod regime may affect carp growth rate, maturation and spawning alacrity. Unlike the situation in the salmonids, however, artificial change of photoperiod is not applied during the carp production cycle. During the main part of the production cycle, when the carps are held in extensive earth ponds, fish are exposed to a natural photoperiod, which is associated with other environmental features, such as temperature. Carp may be subjected to abnormal photoperiod only when moved into the hatchery building for preconditioning before reproduction, but any effect of photoperiod on time to onset of spawning minimal as it is controlled by hypophysation. Hence, any serious consequences for carp welfare can be hardly expected in relation to changed photoperiod.

3.3. Conclusion and research gaps

Light represents an important environmental factor that could influence severely fish behaviour and physiology. Sudden changes in light intensity from the dark to the light phases of the dark/light cycle can induce behavioural stress responses.

There is evidence of photoperiod influence on changes in the immune system in sea bass, as well as in sea bream, while the welfare consequences of artificial photoperiod treatments in salmonids are not yet fully known.

On the other hand, photoperiod is actually considered as one of the most important environmental parameters triggering puberty and reproduction in fish.

4. State of the art on husbandry - Stocking density

4.1. Current regulation

According to Reg. EC 889/2008,

Article 25f (1) "*The husbandry environment of the aquaculture animals shall be designed in such a way that, in accordance with their species specific needs, the aquaculture animals shall: (a) have sufficient space for their wellbeing; (b) be kept in water of good quality with sufficient oxygen levels, and (c) be kept in temperature and light conditions in accordance with the requirements of the species and having regard to the geographic location*".

Article 25f (2) "*Stocking density is set out in Annex XIIIa by species or group of species. In considering the effects of stocking density on the welfare of farmed fish, the condition of the fish (such as fin damage, other injuries, growth rate, behaviour expressed and overall health) and the water quality shall be monitored*".

Article 25h (3) "*Aeration is permitted to ensure animal welfare and health, under the condition that mechanical aerators are preferably powered by renewable energy sources. All such use is to be recorded in the aquaculture production record*".

Article 25h (4) "*The use of oxygen is only permitted for uses linked to animal health requirements and critical periods of production or transport, in the following cases: (a) exceptional cases of temperature rise or drop in atmospheric pressure or accidental pollution, (b) occasional stock management procedures such as sampling and sorting, (c) in order to assure the survival of the farm stock. Documentary evidence shall be maintained*".

Article 25j "*Feeding regimes shall be designed with the following priorities: (a) animal health; (b) high product quality, including the nutritional composition which shall ensure high quality of the final edible product; (c) low environmental impact*".

Most of the references to the regulation reported in chapter 3.1 Water quality, as well as in chapter 3.2 Light and photoperiod, can usefully be considered also in relation to stocking density.

4.2. Current scientific knowledge

Rearing density is normally defined as the weight of fish per unit volume of water and typically refers to the concentration at which fish are initially stocked in a system. Furthermore, crowding is often loosely referred to high rearing density (Ashley 2007; Huntingford et al., 2006).

Rearing density in aquaculture has raised concern with respect to welfare, due to public concern about the welfare of farmed fish. Indeed, rearing density encompasses a complex web of interacting factors, such as water quality, social interactions, fish to fish interaction and fish to housing interaction that can have an effect on many aspects of welfare (Ashley 2007; Turnbull et al., 2008). Huntingford and Kadri, (2008) suggested that a combination of welfare indices e.g. behavioural and water quality monitoring would be a better way to ensure fish welfare in aquaculture than monitoring one index. Other factors to pay attention, when considering the effects of stocking density upon welfare, is the high variability between studies on the same species due to e.g. differences in experimental design (Adams et al., 2007) and also the choice of welfare indicator (Ellis et al., 2002; Canon Jones et al., 2011), which can confound the suitability of the recommendations to be made. Therefore, it is very difficult to make generalisations about how rearing density affects welfare for all situations (Turnbull et al., 2008; Ellis et al., 2002; Conte 2004). However for many teleost high rearing densities induce the increase of the energetic expenditure for basal life functions, that in turn could become detrimental for growth, immune-resistance, and could also affect the social interaction between fish (Huntingford 2004; Martins et al., 2012).

Stocking density in sea bass and sea bream

Under high rearing density both adult and juveniles sea bass grow slower than under low rearing densities (Saillant et al., 2003, Roque d'Orbcastel et al., 2010). There is some discordance about density effects on fish growth. Lupatsch et al. (2010) did not find any significant difference in growth performance and voluntary feed intake between the groups raised at different densities. Notwithstanding, density had no effect on sex ratio, suggesting that the high densities usually applied in aquaculture are not involved in the systematic excess of males reported in farmed populations (Saillant et al., 2003a).

From a physiological point of view, high density condition increases red muscle activity leading to a rise of the global scope for activity (Lembo et al., 2007). In sea bass reared at 50 kg m^{-3}, fish muscle activity, measured as EMG activity with telemetric technologies, was on average twofold higher than in fish at 10 kg m^{-3} (Carbonara et al., 2013). Haematological parameters are indicators of fish oxygen demand to maintain the basal metabolism. Haemoconcentration, indeed, is reported as a strategy for increasing oxygen carrying capacity of blood during periods of high energy demand (Houston 1990), such as a stress event or an important swimming activity. Haematocrit, haemoglobin and red blood cells count have generally the higher levels at the higher densities (Carbonara et al., 2013). Physiological responses to stress is driven by an increase of the plasmatic cortisol levels. There is, indeed, evidence that, between certain limits, the cortisol concentration increases proportionally with the stress levels, just before down-regulation control and saturation of the cortisol receptors occur (Mommsen et al., 1999). Santos et al. (2010) showed that increased density levels reduced feed intake and growth and that feed intake reduction was partially compensated by a decrease in maintenance requirements for energy at the highest density. Another result is shown by Roncarati et al. (2006), regarding intensively reared sea bass. Plasma triglycerids, total cholesterol and transaminases were found to be always significantly higher than in semi-intensively maintained fish.

Farmed fish experience non-natural high densities within the cages, which has also been related with increase of plasma cortisol and differences of fish growth and welfare. Increasing stocking density, resulted also into an exponential increase of the escape rate from cages. Variations on fish interactions towards the net pen were found and were associated with both rearing density and the condition of the net. Particularly, sea bream increased net inspection and biting in relation with the rearing density. Additionally, sea bream was further attracted by damages on the net pens, while individuals were capable of extending existing damages. In particular, limited-fed fish are more attracted by damages on the aquaculture net structure and present higher escape rate (Glaropoulos et al., 2007). Just after a tear was created on the net single individuals shortly initiate escape (Papadakis et al., 2013).

Experiments conducted on sea bream juveniles show that confinement at high density (26 kg m^{-3}) and successive 30 second aerial emersion in a dipnet represent such a stress event that a series of physiological responses are activated at the expense of plasma glucose, lactate and osmolality at any time during confinement or post-handling (Barton et al., 2005). Moreover a reduction in plasma cortisol response to acute handling was also observed. Likely it resulted from negative feedback of mildly but chronically elevated circulating cortisol caused by the stressors on the hypothalamic-pituitary-interrenal axis (HPI axis). The cortisol behaviour suggests a reduction in the gilthead sea bream's normal capacity to respond to an acute stressor.

Montero et al. (1999) described the effects of high rearing density on juveniles sea bream reporting, as a consequence of the stressful condition, an increase of haematocrit, haemoglobin and red blood cell concentration and the decrease of the alternative complement pathway (ACP), an important component of the immune system of fish. This effect, in salmonid species, has been reported to be a consequence of an elevation of plasma cortisol. Indeed, high plasma cortisol levels produce an immunosuppressive effect in fish reducing circulating lymphocytes and increasing the susceptibility of fish to disease. Moreover the authors observed the decrease in hepato-somatic index and an altered liver fatty acid composition. These alterations reflected the effect of stocking density on lipid metabolism channelled to increase the energy demand.

Batzina et al. (2014) showed that sea bream reared at 4.9 kg m^{-3} exhibited growth, aggressive behaviour and size distribution indicating that such low density created a less favourable social environment than specimens reared at 9.7 kg m^{-3}. The author reported also that the use of blue substrate in tank enhanced growth, suppressed aggression and reduced brain serotonergic activity.

Stocking density in trout

Boujard et al. (2002) investigated the effect of stressors, commonly encountered in intensive rearing, on trout held at stocking densities of 25, 70 and 100 kg m^{-3} submitted to different levels of food accessibility, on feed intake, feed utilisation and feeding behaviour. The authors concluded that reduced feed intake and resulting decrease in growth at high density was due to food accessibility, and not crowding stress.

North et al. (2006) studied the impact of the stocking densities of 10, 40 or 80 kg m^{-3} on a variety of physiological and morphometric indicators. They demonstrated that being held at high density (80 kg m^{-3}) did not have consistent effects on growth rates or physiological indicators of welfare, despite increased fin erosion. Furthermore, they found evidence for stronger dominance hierarchies at low density (10 kg m^{-3}). Consequently, it was concluded that both low and high stocking densities had the potential to compromise welfare (North et al., 2006).

A study by Rasmussen et al. (2007) examined the influence of the combined effects of stocking density, fish size and feeding frequency on fin condition and indicators of growth performance in two experiments. The first experiment showed that there was no effect of density (41 and 92 kg m^{-3}) on indicators of growth performance. However, an effect of density and fish size (70 or 125 g) acted together to impair fin condition; the second experiment showed that growth performance was reduced at high density (124 kg m^{-3}) compared to low density (45 kg m^{-3}), but that fin condition was better at high density compared to the lower density (Rasmussen et al., 2007).

The combined effects of stocking density (25, 74 and 120 kg m^{-3}) and water quality (low and high) on indicators of welfare and growth were studied by Person-Le Ruyet et al. (2008). They concluded that growth performance was best under high water quality conditions at all densities and that, irrespective of water quality, growth performance was the lowest at the high density (120 kg m^{-3}) despite not observing any major physiological disturbances (Person-Le Ruyet et al., 2008).

There are two studies that have investigated the combined effects of stocking density (~25 and ~100 kg m^{-3}) and sustained exercise (water current of 0.9 L s^{-1}). The first study showed that high density, irrespective of water current, resulted in a lower growth performance. Furthermore, water current was shown to have a positive effect on energetic budgets, reducing metabolic rate irrespective of density, and was attributed to induce schooling behaviour thereby reducing aggressive behaviour and stress (Larsen et al., 2012). The second study showed that growth rates were reduced at high density, irrespective of water current, and this was attributed to high energy used. The authors concluded that this was unlikely to be due to chronic stress, as cortisol values were low at all densities, but may have been due to an alteration in physiological state (McKenzie et al., 2012).

The overall picture arising from the studies performed to date investigating the effects of stocking density on different parameters suggests that both low and high densities are potentially detrimental to welfare in rainbow trout. Interestingly, what is considered low density and what is considered high density appears to be quite ambiguous, as these ‘definitions’ vary between studies. Furthermore, the results of these studies clearly illustrate the complex nature of the interaction between stocking density and fish welfare, with several environmental factors interacting together and with density to influence indicators of welfare and performance. As a consequence, it is also a complex undertaking to model these multiple interacting and confounding influences of stocking density on measures of welfare (Turnbull et al., 2008), in an effort to gain an overall understanding.

Stocking density in freshwater salmon

Brockmark et al. (2007) looked at the effects of density and environmental enrichment on Atlantic salmon pre-smolts. Looked at four tank treatments (1) high density 3.75 kg m^{-3}; (2) low density 1.35 kg m^{-3} (LD); (3) high density with in-water structure (HDS); and (4) low density with in-water structure (LDS). Around 3,000 fish were distributed in each tank and fish weighed 5 g 3 months after start of experiment. Enrichment was artificial weed like structures. Freshwater data reported up to 311 days after start of experiment. Looked at growth, condition factor and dorsal fin damage. Fish in low density enriched tanks and low density tanks grew better and had better condition factor than those at higher densities. Also had lower mortality rates. Fish in low density enriched tanks and low density tanks had less fin damage than those at higher densities. Enrichment had no effect on fin damage. Enrichment produced salmon with a unimodal size distribution, whereas rearing in conventional tanks leads to a bimodal size distribution.

Cañon Jones et al. (2011) used social network analysis of behavioural interactions and fin damage to assess the effects of stocking density on the welfare of Atlantic salmon parr. Set up eight tanks containing ten ca 110 g parr at densities of either 8 kg m^{-3} or 30 kg m^{-3}. Fish were subject to high density for 10 days out of 30 day period. Fed to feed tables for 30 minutes each day. Looked at growth, condition factor, mortality, aggression and fin damage. Fish grew better, had better condition factor and had lower total aggressive interactions (attacks, displacements and fin bites) at 30 kg m^{-3} densities. Authors state "density therefore has a differential detrimental effect upon performance and welfare depending upon the choice of welfare indicator (e.g. growth and condition vs. aggression and fin damage)."

Hosfeld et al. (2009) looked at effects of fish density on growth in intensively produced Atlantic salmon parr. Distributed 2,940 pre-smolts ca 70 g into eight 500 L tanks at either 21, 43, 65 or 86 kg m^{-3}. "The experimental set-up included independent replicates of each weight group (21 kg m^{-3}: n = 141 and n = 153, 43 kg m^{-3}: n = 282 and n = 305, 65 kg m^{-3}: n = 444 and n = 444, 86 kg m^{-3}: n = 597 and n = 574)." Matched water quality but not group size between treatments. Looked at weight, condition factor, fin damage (dorsal and pectoral fins) and glucose for 100 days. Stocking density had no effect on condition factor. Also had no effect on plasma glucose at the end of the study, but at the mid-point of the study plasma glucose levels were highest in the 86 kg m^{-3} treatment. Stocking density had no effect on growth. Stocking density also had no effect on fin damage after 12 weeks in seawater, but no report on levels during the freshwater experiment.

Stocking density in seawater tanks salmon

Adams et al. (2007) looked at the welfare of salmon held in seawater tanks at differing stocking densities and disturbance levels. Looked at aggression, growth, fin damage to dorsal caudal and pectoral fins, glucose and plasma cortisol. Two year old salmon of 1 kg were held at either 15, 25 or 35 kg m^{-3} for 51 days and fed two meals per day according to feed tables. No effect of stocking density on growth. No effect of stocking density on aggression outside feeding, but post-feeding aggression was highest at 15 kg m^{-3} and in tanks that had the lowest levels of disturbance. Overall, welfare was highest at 25 kg m^{-3} and lowest at both 15 and 35 kg m^{-3}. Also found some fish have poor welfare at all stocking densities measured.

Kjartansson et al. (1988) looked at the effects of stocking density on physiology and growth of tank reared adult Atlantic salmon. "Adult Atlantic salmon, *Salmo salar*, at initial mean size of 1.75 kg, were stocked at three different fish densities, 35 - 45, 65 - 85 and 100 - 125 kg m^{-3}, for 101 and 143 days in circular tanks under controlled environmental conditions including adequate flow-through of oxygenated water." Measured growth, glucose, lactate, haematocrit, plasma cortisol. At day 101, haematocrit was significantly higher in 100 - 125 kg m^{-3} fish; glucose was significantly lower in 35-45 kg m^{-3} fish; lactate was significantly higher in 100 - 125 kg m^{-3} fish. At day 143, no significant difference in plasma cortisol levels between treatments. Density had no significant effect upon growth or condition factor throughout the study. Authors stated "The results indicate that the fish did not experience a high-level state of chronic stress, not even at the high density. Social and size hierarchies were apparently not established at increased densities. The upper density limit for Atlantic salmon post-smolts, in suitable land-based rearing systems, appears to be higher than the highest one investigated here."

Stocking density in seawater cages salmon

Johansson et al., (2006) looked at the effect of environmental factors on swimming depth preferences of Atlantic salmon in sea cages at two different stocking densities. Compared three cages of 7 – 11 kg m^{-3} (1.7 kg adult salmon, ca 26,000 fish per cage) against three cages of 18 – 27 kg m^{-3} (1.7 kg adult salmon, ca 74,000 fish per cage). Looked at swimming depth and density preferences over 3 days. Authors stated “there was a negative correlation between stocking density and oxygen conditions” and “analyses showed differences in behaviour between normal and high stocking densities”.

Oppedal et al., (2011) looked at how ”Fluctuating sea-cage environments modify the effects of stocking densities on production and welfare parameters of Atlantic salmon”. Compared normal (5.6 – 14.5 kg m^{-3}) and high (15.7 – 32.1 kg m^{-3}) stocking densities in triplicate 2000 m^{3} sea-cages for three months. Fish in the normal density groups weighed 1.3 kg (ca 26,000 fish per cage), fish in the high density groups weighed 1.3 kg (ca 74,000 fish per cage). Looked at growth, condition factor, fin damage, cataracts, lesions, feed intake and FCR. Fish under normal density had better condition factor throughout the experiment; better SGR during the last 6 weeks of the study; better feed intake during the last 3 weeks of the study. There were no differences in fin damage or body lesions until the last sample point (more fin damage and lesions in fish under the higher stocking density). Fish under the high stocking density had more cataracts than fish under normal densities from the first to the last sample (there was already a difference between treatments at the start of the trial). Authors stated “These findings clearly demonstrate that salmon welfare was breached beyond an upper stocking density level of 25 – 30 kg m^{-3} under the environmental conditions experienced in this study.”

Turnbull et al. (2005) examined the welfare of cage held Atlantic salmon on a commercial marine farm at densities ranging from 9.7 to 34 kg m^{-3}. 10 month study, looking at three stocking densities (15, 25 and 35 kg m^{-3}). Looked at growth, fin damage to dorsal caudal and pectoral, glucose and plasma cortisol. No relationship between stocking density and welfare up to ca. 22 kg m^{-3}, beyond this point any increasing stocking density was associated with poorer welfare. Authors stated “Our data do not allow us to suggest an alternative single threshold stocking density that will ensure the welfare of the fish concerned, since stocking density combines with other factors that will vary from time to time and place to place, such as the energy of the site and cage deformation due to water flow. Good welfare can be maintained at high densities and that conversely low densities are no guarantee of good welfare.” Further, “There was no significant association between the welfare score and the length of time since grading or lice treatment.”

Stocking density in shrimp and mollusc

To a large extend, shrimp and mollusc rearing are extractive cultures (feed on natural or induced productivity), in which the main part of the feed (shrimps) or all the feed (molluscs) is provided by the environment (Gopikrishna et al., 2011). Therefore, the stocking density considerations are more related to feed availability than to welfare considerations. In shrimp culture, there is a link between density and health, which is directly related to the environment quality through its carrying capacity (Barman et al., 2013; Lemonnier et al., 2007).

Appropriate stocking density for extractive aquaculture should be linked to the carrying capacity of the production area. Carrying capacity is linked to the environmental conditions (trophic capacity, hydrodynamics), to the cultivated species (filtration rates, sizes) and the cultural practices (rearing volume, estimated total biomass, stocking densities) of the area.

In a Mediterranean context, Gangnery et al. (2001) recommended from 1.5 to 2 ropes per m^2 for mollusc productions. Some models are developed to evaluate the carrying capacity of production areas; those models can predict responses in terms of bivalve growth rate in relation to the different management strategies, taking into account biomasses, species and environmental conditions (Heral, 1993). For example, ECASA project (www. ecasatoolbox.org.uk) identified models that can be used to minimize environmental impact from bivalve aquaculture operations.

Stocking density in carp ponds

Stocking levels in a carp pond vary depending on the nature of the pond, whether it is winter or summer and whether there is to be supplementary feeding. The most common fishery practice in pond farming under conditions of temperate climate, however, is to stock fish at a density of 300 to 1,500 kg ha^{-1}. Actually, the real density in the pond will often be much higher in particular areas, because of the carp's propensity for shoaling. There is no evidence that production of carp under such conditions is in any way stressful for carp. Indeed, there is evidence that any significance decrease of stocking density may exert a negative effect on growth rate and increased mortality.

4.3. Conclusion and research gaps

Rearing density encompasses a complex web of interacting factors, such as water quality, social interactions, fish to fish interaction and fish to housing interaction that can have an effect on many aspects of welfare (Ashley 2007; Turnbull et al., 2008). Depending on the type of rearing system and species, the recommendations ranged from 4 to more than 267 kg m^{-3} (Ellis et al., 2002).

Such a wide range of recommendations is in part due to a lack of complete understanding of how the different environmental factors interact with each other and with stocking density to affect welfare (Ashley 2007). Another reason may be that the effect of density measures on welfare may vary greatly between studies due to the study-specific nature of experiments, e.g. studies vary in experimental duration, water quality, density levels used, feeding method, size of the fish, life history of the fish, level of domestication, type of rearing system used and environmental conditions. A density threshold for one set of conditions may, therefore, not be relevant for another (Ashley 2007) and makes comparison of the results between studies difficult. However, for many teleost, high rearing densities have been demonstrated to induce the increase of the energetic expenditure for basal life functions, that in turn could become detrimental for growth, immune-resistance, and could also affect the social interaction between fish (Huntingford 2004; Martins et al., 2012).

It is worth to highlight that most of the experiments on the stocking density reported in the cited literature are supported by the use of oxygen to adapt the water quality to the increased stocking density, which would be not in line with several principles/rules of the organic regulation (e.g. "*… organic production should be as close as possible to nature …*" Reg. EC 710/09, recital 11).

Concerning shellfish, carrying capacity of the production areas has to be evaluated to defined appropriate stocking density for their aquaculture.

5. State of the art on husbandry - Transport, handling and behavioural interactions

5.1. Current regulation

According to Reg. EC 889/2008,

Art. 25h (1): "*Handling of aquaculture animals shall be minimised, undertaken with the greatest care and proper equipment and protocols used to avoid stress and physical damage associated with handling procedures. Broodstock shall be handled in a manner to minimize physical damage and stress and under anaesthesia where appropriate. Grading operations shall be kept to a minimum and as required to ensure fish welfare*".

Art. 25h (4): "*The use of oxygen is only permitted for uses linked to animal health requirements and critical periods of production or transport, in the following cases: (a) exceptional cases of temperature rise or drop in atmospheric pressure or accidental pollution, (b) occasional stock management procedures such as sampling and sorting, (c) in order to assure the survival of the farm stock. Documentary evidence shall be maintained*".

Art. 32a: "*1. Live fish shall be transported in suitable tanks with clean water which meets their physiological needs in terms of temperature and dissolved oxygen. 2. Before transport of organic fish and fish products, tanks shall be thoroughly cleaned, disinfected and rinsed. 3. Precautions shall be taken to reduce stress. During transport, the density shall not reach a level which is detrimental to the species. 4. Documentary evidence shall be maintained for paragraphs 1 to 3*".

5.2. Current scientific knowledge

The transportation of live fish involves the transfer of large numbers (or biomass) of fish in a small volume of water. Some important environmental parameters could severely change during long transportations such as water temperature, oxygenation, CO_2 concentration (Delince et al., 1987). Handling and confined spaces could generate hyperactivity conditions that could result in lactate accumulation and affect blood oxygenation capacity. While fish may be harvested and sold fresh dead, sometime farmers prefer to transport market size fish live from their farms to landing points where the fish are picked up by trucks with tanks. Fish are then sold alive commanding higher prices (FAO 1988).

Stocking density has a large effect on social interactions between fish. This is the passive non-aggressive behavioural interactions, such as collision and abrasion with conspecifics and the physical tank environment, as well as aggressive behavioural interactions between conspecifics that can be detrimental to welfare (Ellis et al., 2002).

There are several detrimental effects of aggressive behaviour on aspects of welfare such as fin erosion, body injury, social stress, loss of appetite, suppressed growth rate, elevated metabolic rates, and disease and mortality (Ellis et al., 2002). Additionally, increased external damage to scales and fins in combination with reduced immune-competence (Olsen and Ringø, 1999) has been attributed as a cause for increased susceptibility to infectious diseases (Pottinger and Pickering, 1992).

Handling salmonids

It is commonly assumed that in salmonids aggression decreases with increasing density. This may be due to the fact that establishing and maintaining ordered dominance hierarchies becomes increasingly difficult at high densities, thereby decreasing the quantity of aggressive acts (Alänärä and Brännäs, 1996). Therefore, by increasing rearing density, the damaging territorial aggressive behaviour can be altered to shoaling behaviour (Grand and Dill, 1999). For example, there is evidence that rainbow trout show shoaling behaviour in intensive culture, which has been shown to reduce aggressive behaviour (Ellis et al., 2002). There is also, evidence for the formation of dominance hierarchies at low densities in rainbow trout (North et al., 2006).

Atlantic salmon in the natural environment have a preference for substrate sizes from 15-260 mm, such as pebbles or cobbles and also a preference for cover (Armstrong et al., 2003 and references therein). Using physical enrichment materials, e.g. by providing substrate or providing shelters to increase habitat complexity, can improve welfare by reducing aggression (Batzina and Karakatsouli, 2012; Batzina et al., 2014) and reducing injuries (Persson and Alanärä, 2014). However, these potential benefits can be both life stage and species specific.

As already presented in the previous paragraph on stocking density, Brockmark et al. (2007) studied the effects of density and environmental enrichment on Atlantic salmon pre-smolts and found that fish in low density enriched tanks and low density tanks grew better and had better condition factor than those at higher densities. The study also showed that “These results collectively indicate that reduced rearing density may be more important than structural complexity for improving post-release performance of juvenile Atlantic salmon”.

Millidine et al. (2006) studied the effect of a shelter on standard metabolic rate of Atlantic salmon. Wild parr were used in pairwise comparisons. The authors stated “The fish without access to a shelter had the higher standard metabolic rate in all but one of the 14 pairs of fish. The magnitude of this difference was substantial, averaging a 30% increase in metabolic costs in the absence of shelter. In all cases the fish in a test pair (shelter/no shelter) with the higher metabolic rate also had the darker coloration.” Also according to Höglund et al. (2000) the dark colouration of the unsheltered fish may be associated with elevated stress levels. Näslund et al. (2013) looked at the effects of tank enrichment upon Atlantic salmon welfare in a 31 week experiment. There were 80 fish per tank, and three treatments: (i) standard barren hatchery environment, (ii) plastic tubes (substrate enrichment), (iii) shredded black plastic bags and the study was carried out to assess fin damage and cortisol levels. Fish from barren tanks had higher levels of cortisol than those from enriched tanks. No significant difference between enrichment types. Fish from barren tanks had higher levels of fin damage than those from enriched tanks.

Pickering et al. (1987) looked at overhead cover on the growth, survival and haematology of juvenile Atlantic salmon. They reported that access to overhead cover improved growth and reduced chronic stress response (thrombocytopenia and lymphocytopenia). Mortality was not affected by the presence of tank cover.

As regard stocking densities and disturbance levels, fin damages, environmental enrichment and substrate needs, see also references to Adams et al. (2007), Turnbull et al. (2005) in the previous paragraph on the Stocking density.

Transport and handling sea bass and sea bream

Exposure of fish to aquaculture practices such as handling, crowding and transport stimulates a stress response which starts with plasma catecholamines and corticosteroids, changes in features related to metabolism, hydromineral balance, and cardiovascular, respiratory and immune functions (Barton 2002). The catecholamines and corticosteroids production are also responsible, at cellular level, for the expression of heat-shock or stress proteins. Indeed, in adult sea bass exposition to transport stress causes the HSP70 proteins production at skeletal muscle levels (Poltronieri et al., 2009).

Another important cellular response was described by Vazzana et al. (2002) in sea bass exposed to confinement stress. The authors showed that high levels of plasmatic cortisol and glucose are correlated with the suppression of cytotoxic activity affecting the eosinophilic granule cells of the peritoneal cavity. This result is in accord with the already known immunosuppressive effect of increased plasma cortisol levels during stress events. On the other hand, exposition to handling is responsible of the activations of the brain–sympathetic–chromaffin cell (BSC) axis and of the brain–pituitary–interrenal (BPI) axis in sea bream (Arends et al., 1999). In particular, 3 min exposition to air caused, within 30 min, an increase in plasma concentrations of cortisol, glucose, lactate, α-melanocyte stimulating hormone (α-MSH) and osmolality. Sea bream confinement at a density of 70 kg m^{-3} generated the cortisol, ACTH and α -MSH increase within 1 h.

Handling stress is known to generate in sea bream a diffuse immune system alteration. Sunyer et al. (1995) showed that sea bream exposed to repeated handling procedures were characterized by lymphocytopenia and decreases in hemolytic activity, agglutination capacity, and antibody titer.

Freshwater transport of juvenile salmon

Iversen et al. (2005) looked at stress responses in smolts during commercial well boat open-hold transports. They monitored plasma cortisol, glucose and lactate before and after five well boat transports. The weight of fish was 70-138 g, densities during transport were 17 - 40 kg m^{-3}. Duration of transport was 4 - 40 h. The loading process was suggested to be more severe a stressor than transport per se. Only minor plasma cortisol increases were noted during unloading and in 80% of the transports plasma cortisol levels had returned to the resting level at the time of arrival. The fifth transport had high levels of cortisol at unloading and also demonstrated increased mortality after transfer. Duration of transport had no effect on cortisol. Glucose and lactate were not consistently affected by handling and transport. Authors suggested “well boat transports seemed to have an important recovery function”.

Nomura et al. (2009) looked at the physiology of smolts during commercial land transport. Smolts (70 - 100 g) were transported at a density of 68.4 ± 14.6 kg m^{-3}, oxygen levels were maintained at 13 – 15 mg L^{-1} for road trips of 30 - 90 min duration. The authors studied plasma cortisol, glucose, lactate, potassium, sodium and chloride concentrations. Plasma cortisol levels had significantly increased after transport compared to controls. No effect of transport on glucose, lactate, chloride, sodium or potassium levels was found. Authors stated “primary and secondary stress responses were detected in smolts after truck transport, but these were moderate when compared with maximum levels cited in the literature for fish.”

Seawater transport of post-smolts and adult salmon

Prior to slaughter (normally 1 - 3 days) the salmon are transported with well boats from the on-growing farm to the slaughter house where they are placed in waiting cages just within immediate adjacency to the slaughter house. At slaughter the fish are crowded inside the waiting cages to facilitate pumping to the slaughter house. During crowding the net is tightened to increased fish density normally up to 300 kg m^{-3}. The crowding increase stress, shown as increased behavioural panic, lactate and cortisol (Espmark 2005) and may also cause injuries caused by the high density. Other publications have also shown increased stress and reduced welfare because of crowding (e.g. Skjervold et al., 2001, Roth et al., 2012). Another concern with waiting cages is the poor water quality that often results from the location of the cages. Since they are located close to the slaughter house they are, as opposite to the on-growing cages, located close to shore where up-whelming, and circulation of fresh oxygenated water is poor (Espmark et al., 2012). The levels of oxygen during crowding may become critically low, and oxygenation should be carried out.

After crowding the fish are often pumped with vacuum pumps into the slaughter house. Pumping may also cause stress/injuries to the fish (Roth et al., 2012). High pumping speed, collisions with conspecifics, walls and valves may stress and harm the fish (Espmark et al., 2012). Also pumping heights cause stress in fish, however because the negative effect of and focus on heights, farmers now place the pumps as low as possible and preferably close to the sea level.

Erikson et al. (1997) looked at short term transport (1.5 h) of ca 25 tonnes adult salmon (average weight 5.1 kg) by well boat at a density of ca 125 kg m^{-3}. They measured water temperature, dissolved oxygen, and pH during transport in addition to carbon dioxide, salinity (S), total alkalinity (TA) and total ammonia nitrogen. They also used white muscle pH, redox potential, PCr, ATP, struggle-induced IMP and mean AEC values as indicators of handling stress. No major effects of handling stress were found. No mortality during transport either. The authors hypothesised that this was due to “good seawater quality during transport, to a quick bulk netting of the fish from well-boat to the slaughter line”.

Farrell, (2006) assessed welfare of adult salmon under sea transport to the processing plant. The transport parameters included an 11 h journey, 11,002 fish and a total biomass of 62,132 kg (average fish, 5.6 kg), the fish were held at ca 95-100 kg m^{-3} density. The oxygen levels in the outflow were maintained > 7 mg L^{-1} (with additional oxygenation where required), after an initial ca. 30 minute drop to ca 5.5 mg L^{-1} during loading of the fish. 26 fish died during transport and 3 days post-transport, equating to 0.04% total mortality. The study also assessed welfare using bulk metabolic rate (bulk oxygen uptake) and reported that fish recovered quickly from the loading stress using bulk metabolic rate data. Also corroborated these results by behavioural observations of fish facing water flow and slowly schooling whilst exhibiting light ventilation of the gills. Concluded 11 hours of ca. 100 kg m^{-3} density transport was no welfare risk for large salmon.

Gatica et al. (2010) assessed the effects of open-hold seawater transport by well boat on fish physiology and welfare. The experiment was based upon three transport trips with an average well density of 107.8 kg m^{-3}, average water temperature of 11.5°C, 8 h duration and an average 18,700 fish transported during each trip. 10 fish were sampled at i) on farm, ii) after loading, iii) after transport in the well boat, iv) after unloading, v) after resting and vi) after pumping to the processing plant. Welfare was assessed using blood glucose, lactate, cortisol, sodium, chlorides and osmolality. The results suggest the most stressful stage of the transport and pumping process was during pumping from the resting cages to the processing plant. There were no significant effects of loading into the well boat on any measured blood parameter, and only glucose levels significantly peaked during transport, the rest mostly peaked during pumping from the resting cages to the processing plant.

Nomura et al. (2009) looked at the physiology and behaviour of smolts during open-hold commercial sea transport using a well boat. They studied plasma cortisol, glucose, lactate, potassium, sodium and chloride concentrations. Behavioural indicators were included: i) Fish orientation, ii) fish density (nearest neighbour distance, NND) was used as a proxy for fish density iii) swimming effort (tail beat frequency) and iv) over erratic behaviour. The authors studied six transports of ca 2.5 h duration and involving 135,000 to 210,000 fish for each transport. Authors stated "The benefits of a live-haul vessel with flow-through holds were demonstrated by the physiological and behavioural parameters either recovering towards or being fully recovered to control levels".

Tang et al. (2009a) also assessed welfare of adult salmon under sea transport via well boat. And looked at data from 45 live-haul transports that lasted >10 h. Densities during transport were 63-150 kg m^{-3} and the fish weight across all trips was 5.13 ± 0.47 kg. Welfare was assessed using bulk metabolic rate (bulk oxygen uptake). Authors reported that fish recovered quickly from the loading stress using bulk metabolic rate data. Recovery of bulk oxygen uptake during transport was completed by 6.5 h and the highest rates of bulk oxygen uptake were recorded 1 h after the transport started, suggesting loading has a marked effect on stress. The authors also reported that densities of 63 - 150 kg m^{-3} did not significantly affect bulk oxygen uptake, suggesting this range does not stress adult salmon sufficiently enough to affect this parameter.

In another study, Tang et al. (2009b) assessed the welfare of adult salmon under closed-hold sea transport via well boat. The authors carried out six 30 minute closed-hold experiments on 13,000 - 15,000 fish at an average fish density of 135 ± 4 kg m^{-3}. Fish weight was 5.7 ± 0.2 kg, water temperature was 10.6 ± 1.2°C. During closed-hold 30 minute period, CO_2 increased from 1.2 mg L^{-1} to 5.3 ± 0.2 mg L^{-1}, well below threshold levels. The authors concluded that "closed-hold transport for limited periods of time can be accomplished at the typical densities and masses involved in commercial live-haul of adult Atlantic salmon without seriously compromising fish welfare".

Shrimp handling and transport

It was concluded by Sneddon et al. (2014), that the stress-induced avoidance in crustacean is similar to vertebrate anxiety and indicates the ability of invertebrates to exhibit a state similar to mammalian emotion. As to mollusks, the same authors focused on cephalopods but did not touch the question of other mollusks (oysters or others). Some authors worked on the physiological responses of crustacean to different transport practices (Fotedar et al., 2011; Lorenzon et al., 2008; Salin et al., 2001), in order to define codes of practices and defining guidelines for crustacean handling and transportation, mainly to maximize the survival rate and the quality of the product.

A special concern about eyestalk ablation for reproduction in shrimp was pointed out as an important aspect as most shrimp to routinely develop mature ovaries without this practice. It means that without ablation, shrimp hatcheries would have to rely on natural breeding. This is slow and unpredictable, especially for species like giant tiger prawn, therefore it would lead to shortages of the small shrimp needed to stock ponds. The aim of ablation, under these circumstances, is to stimulate the female shrimp to develop mature ovaries and spawn. Even in conditions where a given species will develop ovaries and spawn in captivity, use of eyestalk ablation may increase total egg production and increases the percentage of females in a given population that will participate in reproduction.

There are four main techniques used for eyestalk ablation: pinching, enucleation/slitting, cauterisation and ligation. The EGTOP conclusions were that without eyestalk ablation, production of juveniles is unpredictable and does not allow a guaranteed production cycle. The alternative of collecting breeders in the wild, in absence of a well-documented management plan, is not desirable. Nevertheless, organic principles and consumer expectations are that organic animal husbandry avoids mutilations in all animals. Therefore, for the sake of integrity of organic production, this fundamental principle should be uniformly applied for all animals. However, in case of derogation of such principle, the technique of ligation would be more acceptable than pinching, enucleating by slitting, cauterisation or other methods.

Concerning shellfish transfer activities, Muehlbauer et al. (2014) showed all countries involved in the cultivation of bivalves along the European Atlantic coast are currently conducting transfer activities, in different ways and varying quantities. All life stages from larvae to mature individuals are concerned, and all types of production sites (reared or wild fields). This is particularly true for France and Spain which have a long bivalve cultivation tradition, with permanent transfers between the Atlantic and Mediterranean coasts. Cranford et al. (2012) recommended good husbandry and biosecurity practices essential to successful prevention and control of diseases.

Methods to alleviate exposure to stressors during transport

Foss et al. (2011) looking at the physiological effects of live chilling salmon, measured blood sodium, potassium content, pH, lactate, cortisol and glucose levels as welfare indicators. They reported that a drop in temperature from 16 to 4°C, over 1 h, and a drop from 16 to 0°C, over 5 h, did not affect plasma lactate levels in 300 - 600 g adult salmon. Authors stated "A temperature drop from 16 to 4°C in 1 h or from 16 to 0°C in 5 h does not seem to affect fish welfare in the short term".

Iversen and Eliassen, (2009) looked at the effects of AQUI-S 5.0 mg L^{-1} (isoeugenol 2.5 mg L^{-1}) sedation on the primary (plasma cortisol), secondary (osmoregulation), and tertiary (mortality) stress responses of Atlantic salmon smolts during transport and transfer to sea. 240 smolts, ca 55 - 60 g weight were equally distributed into two 1m3 freshwater tanks. Water levels were rapidly reduced to create densities of 140.5 kg m^{-3} for 20 min. Fish were then hauled into two separate transport tanks of 546 L, with average density of 14.4 kg m^{-3}. Fish were then transported by truck for 2 h and transferred into a single 1 m^3 tank containing full strength seawater. Control fish had significantly higher plasma cortisol levels than sedated fish, for up to 6 h after transport. Cortisol levels were significantly higher than pre-transport levels in both groups, until 12 h after transport. Control fish had significantly higher lactate than sedated fish, up to 1 h after transport, and significantly higher plasma magnesium levels than sedated fish, up to 168 h after transport. Mortality was 11.3% for control fish and 2.5% for sedated fish and ceased 16 days after transport. Authors concluded "that AQUI-S shows promise as a stress-reducing sedative for Atlantic salmon smolts and if used properly could improve animal welfare and survivability during and after common aquaculture related incidents".

Iversen et al. (2009) also demonstrated similar results to above when using 4.0 mg L^{-1} clove oil (90-95% Eugenol).

Harvesting, transport and handling of carp and pond fishes

The process of carp pond harvesting is one of the most important issues of pond farming as far as the fish welfare concerns. Pond harvest is the final step in pond fish culture and hence, it is of extremely high importance with respect to production results and farming success. Many types of fishing equipment are used for catching fish out from ponds depending on the pond type, fish size, species and category. They can be lift nets, cast nets and seine/drag nets. Catching of fish can be done for different purposes, such as sampling, cropping/selective fishing or harvesting.

When sampling, the objective is to catch that quantity of fish which represents the entire fish stock in a pond. For sampling advanced fry, a lift net can be used, while the elder age groups are sampled with a cast net or with a seine net. Sampling of fish is often done by attracting fish with feed. Sampling at the locations of feeding is also widely practiced.

Cropping (partial harvest) or selective fishing is done during the production season without lowering the water level of the pond. Fish are attracted with feed in order to congregate. However, the portion of feed used to attract fish should be much less than the usual feeding ration because fish captured and congested in a net may die if had a full digestive tract.

The entire fish stock is captured from a pond during harvesting. It can be done only together with the partial or total drainage of water. For the sake of easier harvesting, internal or external fishing pits are constructed and used. The internal fishing pits are the lowest water drainage structures of the ponds. The bottom of the external harvesting pits should also be deeper than the other parts of the pond. As the water level decreases fish concentrate there and therefore can be captured quickly and easily.

For partial or full harvesting of advanced fry, 10 – 20 m long and 2.5 – 3.5 m deep nets are used. They are made of light but strong water-resistant material which have netlike patterns and a mesh size of about 2 – 4 mm. For catching the larger age groups, nets should be mounted from factory-made net materials. It depth and length should be approximately 1.5 multiple of the fishing pit dimensions. Also, the net mesh size must be thoroughly selected with respect to fish size and category. The type of the net material is very important with respect to fish welfare. It should be knotless; otherwise, it will wound the skin and scales of captured fish. These wounds are potential entry points for later infections.

During harvesting, fresh water inflow must be provided into the fishing pit with fish concentrated in the net and into the manipulation vats to maintain the oxygen concentration at an appropriate level. Also, fish grading and transportation must be performed with principal requirements for the welfare issues – i.e. in sufficient amount of water with aeration or oxygenation.

Suspended matter is a serious potential problem during harvesting when fish are crowded and disturbed. Suspended solids concentration in the water can increase to >1000 mg l^{-1}. In such cases severe gill obstruction occurs and there is considerable reduction in respiratory capacity, limited gas exchange across the gills and temporary anoxia. Thus, the appropriate water flow is essential during the period of harvesting whether for market or simply to move fish to another pond, as otherwise the suspended solids generated by the close confinement and disturbance of the sediments leads to choking and suffocation of the concentrated fish.

Pond fish are usually transported alive within a fish farm, between farms and to the market. Transport within the fish farm should be done just as carefully as the long-distance transport between farms, i.e. overloading of the transport tanks should be avoided, even if the duration of transport is short. This will reduce stress and suffocation of fish. Special attention must be given to the danger of the temperature shocks which may happen frequently during fish handling and transportation.

Clean, oxygen-rich water and enough space in the transport tanks have to be ensured. There is negative correlation between the temperature of transporting water and the number and total weight of fish transported in a unit volume of water. As the duration of transport increases, the number of the transported fish should be decreased. In order to reduce excess consumption of oxygen, stress and mortality, fish should be prepared for long-distance transport. Keeping them in cages or tanks supplied with current water for a period of about 12 to 24 hours ensures that the digestive tract of fish empties; therefore, they will not contaminate the water of the transport tank with metabolites (harmful gases and liquids) and faeces (harmful materials). During transport, the state of fish and the oxygen diffusion should be checked frequently in order to discover and repair possible problems.

Wintering of fish

In the countries of Central and Eastern Europe, the water temperature of ponds is low in winter and therefore the fish hibernate. The period of wintering is considered as one of the most important parts of the production cycle as the fish welfare concerns. Generally, there are three options for wintering fish. The first option is to leave the fish in the rearing pond for the winter and harvest them in spring; the second is to keep the fish in special wintering ponds; and the third is stocking the fish in the pond in autumn where they will be reared the next season.

When fish are transported into wintering ponds they should be selected by species and size. This facilitates easy handling and stocking or selling of stored fish. The water surface area of wintering ponds varies between a few hundred and a few thousand square meters. The water depth in the wintering pond should be between 1.8 – 2.5 m. It is suggested to keep the wintering ponds dry for at least a few months before they are used for storing fish in order to complete the mineralization of organic materials. The bottom of wintering ponds should be cleaned and disinfected using evenly distributed powder or a solution of lime.

Wintering ponds should have a continuous supply of fresh oxygen-rich water in the rate of 60 – 120 L min^{-1} tonne^{-1} of fish. In order to avoid overhibernation of fish, the water of wintering ponds should not be changed at the bottom. The current of fresh water should run over the fish hibernating at the bottom. This can be done by opening the water outlet at a certain distance (at least 30 – 35 cm) from the bottom. This ensures that the water temperature will remain around 4°C at the bottom. The oxygen content of arriving water can be increased if water is trickled. When water is recycled in the wintering ponds, the oxygen content of the water must be checked daily. If ice develops on the surface of pond water, it is important to clean it, as well as to cut large holes to ensure a way for light and air to enter into the water.

To prevent fish weight loss during wintering, when the water temperature is over 5°C, carefully monitored and well-dosed feeding with pellets may be tried in order to reduce these losses. It has to be ensured that predatory species receive enough food fish during wintering. For this reason, about 10 percent of their total weight in food fish should be stocked together with them.

Predators

In addition, fish predators such as cormorants, herons and otters cause high economic losses to carp pond fish farmers although in some countries they are compensated under law for losses caused by protected animal species. However, their impact upon carp pond stocks does not consist only in predation of fish but they represent an important fish welfare issue.

Despite the numbers of cormorants, *Phalacrocorax carbo sinensis,* in recent years slightly decreased, the migrating flocks of tens of thousands of birds cause direct losses by predation (Adámek et al., 2007) and subsequently strong stress to pond fish (Kortan et al., 2008, 2011a,b) when spending a few weeks on the ponds in spring and autumn as they pass through. Farmers usually are not concerned about a few hundred locally nesting cormorants but numerous populations migrating from Northern Europe to the South consume huge amounts of fish on the Central European ponds as a cormorant eats 0.5 kg fish per day on average.

Recently, also numerous occurence of the Eurasian otter (*Lutra lutra*) on carp ponds leads to significant losses on their stocks (Kortan et al., 2007). The losses do not consist only of direct predation which is often performed, but over-hunting when training youngs in fish capture (Adamek et al., 2003). The problems associated with fisheries management and otter interference are of increasing importance in Central Europe due raising otter population density and re-colonisation of biotopes where they had formerly occurred. These trends are particularly pronounced in fishpond regions (Kemenes and Nechay, 1990; Bodner, 1995).

5.3. Conclusion and research gaps

Based on the Reg. EC 710/2009, the Danish authorities on inspection of organic aquaculture facilities have prepared a guideline for transport of live fish (Larsen 2014), summarized below. Live fish shall be transported fulfilling the physiological needs of the fish with respect to oxygen, i.e. 65 – 120% saturation. The temperature of the water in the transport basins should be the same as the water temperature in the tanks where the fish were reared. The fish shall be starved for a certain period (4 – 10°C days) dependant on water temperature and fish size before transport, i.e. the stomach shall be empty to minimize metabolism and production of metabolites (NH^{4+}) and particulate matter, that will deteriorate water quality, deposits in the gills and further stress the fish. The maximum stocking density allowed is 150 kg m^{-3} and duration of time placed in transportation tanks without water exchange must not exceed 6 hours. Water exchange shall exclusively be taken directly from an approved bore hole or spring. Total retention time in transportation tanks must not exceed 12 h. Total transportation time in tanks and concomitant storage in tanks at the slaughter should not exceed 24 h. In case of transportation by boat, exchange water shall be pumped from a distance of at least 500 m from possible point sources to pollution.

Water can be cooled during transport to alleviate the need of the fish for oxygen and to reduce ammonia production (Lines and Spence, 2012). Freedom Foods Welfare Standard for farmed salmon transport state i) the maximum chilling rate should be 1.5°C per hour, ii) the maximum permitted drop in temperature should be no more than 50% of ambient temperatures at the start of chilling within 24 h, and iii) minimum temperatures at the end of chilling should be no less than 4°C.

Debio Organic Aquaculture Standard for farmed salmon transport and Freedom Foods Welfare Standard state “As a minimum the oxygen content in the water shall be at least 7 mg oxygen per litre.”

Freedom Foods Welfare Standard for farmed salmon road transport suggest maximum stocking densities of 60-100 kg m^{-3}, whilst for salmon transported by well boat suggest maximum stocking densities of 40-50 kg m^{-3}.

Debio Organic Aquaculture Standards suggest: live fish can be transported for a maximum of 6 h by truck. Without water exchange. Max density with transportation of fry is set at 10 kg m^{-3}. There can be at most 30 kg m^{-3} in closed well boat transportation. Well boat with constant water exchange can at most have a fish density of 50 kg m^{-3}.

Besides the above recommendations obtained by some private standards, other possible suggestions could be derived by the pertinent scientific literature cited, such as:

- Monitor CO_2 levels in the water during closed transport (Tang et al., 2009b);
- Monitor the behaviour of the fish during transport using camera’s (Farrell, 2006; Nomura et al., 2009; Martins et al., 2012);
- Using isoeugenol or eugenol for sedation during transport;
- Using physical enrichment materials and providing fish with access to nature-like substrates, e.g. by providing substrate or shelters to increase habitat complexity, might improve welfare by reducing aggression.

For shellfish, as organic farms shall minimise risks to species of conservation interest, transfer of shellfish has to be controlled, to avoid the risk of alien, translocated species or diseases introduction. Risk assessment methodologies could be applied to minimize the impact of transfers and to prevent the introduction of invasive species. An example of a practical plan for shellfish farmers including advice on hygiene, biosecurity and good husbandry practices is provided by Fraser (2010).

6. State of the art on husbandry - Slaughter

6.1 Current regulation

According to EC Reg. 889/2008, art. 25h (5) ”*Slaughter techniques shall render fish immediately unconscious and insensible to pain. Differences in harvesting sizes, species, and production sites must be taken into account when considering optimal slaughtering methods*”.

6.2 Current scientific knowledge

An optimal slaughter method should render fish unconscious and insensible until death, without avoidable excitement, pain or suffering prior to killing. Welfare evaluation at time of slaughter is difficult to perform because it requires a multidisciplinary approach examining various indicators such as brain functions, endocrine responses, behaviour and post-mortem tissue biochemical condition (Poli et al., 2005).

Unconsciousness is defined as and is measured by:

- Unconsciousness is a state of unawareness in which the brain is unable to process sensory input (e.g., during (deep) sleep, anaesthesia or due to temporary or permanent damage to brain function). To prevent recovery, a stunned fish should be insensible. Insensible is defined as the inability to perceive (and as a consequence respond to) stimuli (Van de Vis et al., 2014). The most secure method to determine unconsciousness and insensibility is to observe brain activity using electroencephalogram (EEG) measurements (Kestin et al., 2002).
- For field observations (i.e., during slaughter on a commercial farm or in a harvest facility), registration of EEGs and ECGs might not be feasible. In this case, observation of behaviour can be used. Spontaneous behaviour (e.g., righting response and escape behaviour), responses to stimuli, and physical reflexes (vestibulo-ocular reflex, also known as the eye roll) can be used as preliminary behavioural observations to evaluate loss of consciousness and sensibility in fish (Van de Vis et al., 2014). However, caution is needed when using behaviour and physical reflexes to determine the effectiveness of stunning methods in practice.
- Convulsions can be observed in effectively stunned fish and should not be a cause for concern, provided that a stunning method is applied correctly. To assess whether or not consciousness and insensibility are lost immediately, for electrical stunning the electricity should be applied for 0.5 to 1 s, provided that sufficient current is passed through the brains. Subsequently, measurements should be performed to assess whether or not consciousness and sensibility where lost immediately.
- Stunning is the process that renders an animal unconscious and insensible without causing avoidable stress and discomfort prior to death for a sufficient period of time to allow killing.

Slaughtering fish

Slaughter is defined as the killing of animals, especially farmed ones, for the production of food (Van de Vis et al., 2014). To protect welfare of fish at slaughter, these animals should be stunned prior to killing.

The biochemistry of the muscle post-mortem and the onset of rigor are influenced by the method used in pre-slaughter handling, stunning and killing of fish (EFSA, 2009; Lowe et al., 1993) which, in turn, can compromise the sensory quality and marketability of the final product.

Handling of fish prior to slaughter

Handling of fish prior to slaughter comprises, depending on the fish species, the following steps: fasting, crowding, loading a transport vehicle, transport within a company or between companies, unloading the fish at slaughter house or facilities and lairage (temporal housing) of the animals. During pre-slaughter handling operations, crowding and confinement represent unavoidable practices, required to rapidly remove fish from rearing units.

Fish welfare can be strongly affected by pre-slaughter crowding conditions, since vigorous movements are induced for several minutes before death as well. The time necessary to reach irreversible unconsciousness, muscle pH and rigor state is proved to vary significantly depending on the pre-slaughter and slaughter procedures adopted (Bagni et al., 2007). Osmolality, glucose, lactate and cortisol were used as stress indicators.

Pre-slaughter stress, combined with killing methods involving greater physical activity prior to death, leads to the consumption of the glycogen energy reserve ATP's expense. At the same time, the vigorous swimming during crowding implies an intense use of white muscle. Anaerobic glycolysis increases lactic acid production, causes lower muscle pH and changes the time to onset and resolution of rigor mortis. Moreover, lactic acid is usually further increased by a passive production after death.

Fasting

At the farm, the market-size fish are fasted to empty the gut. An empty gut during these operations benefits the fish by lowering their metabolic activity and so reducing the rate of ammonia and carbon dioxide build-up in the water during crowding and transport (Algers 2009; Ashley 2007). The duration of fasting necessary to empty the gut is species and water temperature dependent but may be expected to be from 1 to 5 days. Food withdrawal for this period is unlikely to cause significant welfare problems (Lines and Spence, 2012).

Long fasting periods (months) were used in the past under the assumption that increasing the starvation period would reduce fat at the muscular level (Einen and Thomassen, 1998; Rasmussen et al., 2000) and eliminate unpleasant flavour. The cumulative stress caused by prolonged fasting leads to immune depression, which makes fish more susceptible to stress mediated diseases during the pre-slaughter period (Algers et al., 2009). There is evidence that in sea bream longer fasting leads to a shorter shelf-life of the product. Indeed, the shelf-life was estimated to be 16 days for sea bream starved for 24 h, 15 days for those starved for 48 h and 14 days for those starved for 72 h (Álvarez et al., 2008).

In sea bass long fasting periods could induce a decrease of the intestinal microvilli length with change in permeability of intestinal mucosa to amino acids. This induces also loss of weight and condition, loss of intestinal fats and plasma protein, together with a precocious involution of gonad tissue, without any variation in the chemical composition of muscle (Algers et al., 2009).

Crowding

Prior to loading a transport vehicle (i.e. a truck, well-boat or aircraft) fish are crowed in cages or tanks. By crowding the density is increased temporarily to facilitate the transfer of the fish to a transport vehicle. Excessive crowding may overtax the animals due to injury and stress combined with low levels of oxygen in the water should be avoided.

During crowding in sea cages the net is tightened to increased fish density normally up to 300 kg m^{-3}. The crowding increase stress, shown as increased behavioural panic, lactate and cortisol (Espmark 2005) and may also cause injuries caused by the high density. Other publications have also shown increased stress and reduced welfare because of crowding (Skjervold et al., 2001, Roth et al., 2012).

In tanks, fish are crowded by e.g. lowering the water level or by limiting the available space for the fish by using a net or a screen. After crowding, the fish are often pumped with vacuum pumps into a vehicle for transport. Pumping may also cause stress and/or injuries to the fish (Roth et al., 2012). High pumping speed, collisions with conspecifics, walls and valves may stress and harm the fish (Espmark et al., 2012). Also pumping heights cause stress in fish, however because the negative effect of and focus on heights, farmers now place the pumps as low as possible and preferably close to the level of the water in the cages or tanks.

Negative effects of crowding and pumping is also documented by Terlouw et al. (2008) and Lines and Spence, (2012).

Loading a vehicle

At a farm fish are transferred to a vehicle by pumping or netting. Care should be taken to prevent injury and stress to fish during loading. Excessive weight loading on fish at the bottom of nets and brailles should also be avoided (Conte 2004; HSA 2005). Moving water along with the fish should cause fewer injuries and appears to be the least stressful technique (FAWC 1996). For more information about pumping, see the previous section. During loading the fish should not be exposed to a fall.

Transport

Transport of live fish is a regular practice on many fish farms, used following harvest, during grading or sorting, to take fish to short-term live storage, to stock ponds in the same or other farms for breeding or growing; or to bring live fish to market. The time of transport varies according to the distance to be covered and the methods being used; on the farm the transport time is usually very short (few minutes) or short (up to 30 minutes). Outside the farm the transport time is habitually longer, varying from a few hours to one or two days (Dalla Villa et al., 2009).

Prior to slaughter (normally 1-3 days) the salmon are transported with well-boats from the on-growing farm to the slaughter house where they are placed in waiting cages just within immediate adjacency to the slaughter house.

Issues that are relevant for loading also apply to unloading.

Lairage

After unloading of a transport vehicle, fish are kept temporarily (lairage) until the process of slaughter commences. Pumping or netting used for unloading may compromise welfare, as described previously. A concern with waiting cages is the poor water quality that often results from the location of the cages. Since they are located close to the slaughter house they are, as opposite to the on-growing cages, located close to shore where up-whelming, and circulation of fresh oxygenated water is poor (Espmark et al., 2012). The levels of oxygen during crowding may become critically low, and oxygenation should be carried out. When fish are kept in tanks measures should be taken to ensure that the water quality does not deteriorate during lairage.

Stunning and killing

In a report from EFSA (2009) it is concluded that stunning either percussive or electrically is the most humane method, and that pre-slaughter treatment as crowding and pumping may cause harm to the fish. When percussion is applied, it should be measured whether the air pressure, which drives the bolt, is sufficiently high to induce immediate loss of consciousness and sensibility (Van de Vis et al., 2014). The EFSA report is a valuable review (EFSA 2009) and should be taken into consideration. Also Southgate and Wall, (2001) summarizes the slaughter and pre-slaughter process.

In sea bass and sea bream, field recognition for unconsciousness or death includes absence of breathing and opercular movements, eyes fixed, absence of response to painful stimuli (pin-prick) and loss of balance (Algers et al., 2009).

The onset and development of rigor mortis is widely used as indicator of pre-mortem stress and is influenced by many factors, such as species, age and size of the specimen, pre-slaughter procedures (Lowe et al., 1993; Nakayama et al., 1999). Bagni et al. (2007) reported that rigor starts earlier in crowded fish. Indeed, the post mortem metabolism varied considerably between stressed and unstressed fish, where ATP is more or less depleted, respectively (Berg et al., 1997).

There are different methods used to kill fish. Live chilling in ice-water slurry was reported as the more common method, while asphyxia (exposure to air) is a rarely used method (Algers et al., 2009).

Electrical stunning and exposure to water saturated with gas mixtures (carbon dioxide and nitrogen alone or as mixtures) have been tested experimentally on fish farms.

In sea bream immersed in an ice slurry the response to handling and breathing all ceased only after 15 – 20 min, whereas carbon dioxide-stunned fish appeared dead after 5 min (Giuffrida et al., 2007). Gilthead sea bream group having slower ATP depletion also showed lower lipid oxidation of muscle during storage. Body temperature decreased faster in liquid ice than in conventional ice. Indeed, the fish slaughtered with liquid ice showed better texture and freshness characteristics. Hence, this method results to be effective and faster than conventional ice, fish may be stressed less as and the method is easily adaptable to the farms needing (Urbieta and Ginés, 2000). However the lowest temperature attained by the liquid ice caused the appearance of cloudy eyes that significantly reduces the commercial value of the fish (Huidobro et al., 2001).

For gilthead sea bream, neither asphyxia in air nor transfer of the fish to an ice slurry are considered to be welfare-friendly: the methods do not induce immediate brain dysfunction and vigorous attempts to escape occur. Percussive and electrical stunning are considered alternatives (Van de Vis et al., 2003).

Bagni et al. (2007) showed that sea bass asphyxiated in air live longer struggle than those killed in chilled water, independently whether they are crowded or not during pre-slaughter. Asphyxia in air leads to a much more prolonged pre-mortem activity in comparison with asphyxia in chilled water, thus severely affecting fish welfare. Anyway, both ordinary killing methods result to be highly stressful.

CO_2 narcosis and ice asphyxia are two common capture procedures in commercial farms. They were demonstrated to produce low muscle pH values (Acerete et al., 2009). CO_2 treatment showed lower lactate and cortisol response compared to asphyxia in ice, while the latter produced a 5-fold increase in glucose levels and a 8-fold increase in cortisol levels.

Electrical stunning can induce immediate loss of consciousness and sensibility in fish. However, reported data show that fish cannot be killed by the use of electricity, as the fibrillation of the heart is not permanent (Van de Vis et al., 2014). This implies that electrical stunning should be followed by a killing method to avoid recovery of the stunned fish. Because stunning and killing are procedures that take some time, it is normally necessary to apply the electrical current not only at a certain, but also for a certain duration of time, so as to allow subsequent killing before the fish have recovered.

For example, Nile tilapia can be stunned using an electrical stun lasting for 5 s and the unconscious and insensible fish can be killed subsequently by chilling in ice water slurry (Lambooij et al., 2008). To prevent recovery of an electrically stunned fish, an effective killing method should be applied. Gill-gutting is not always an effective method, as in an experiment with electrical stunning of Atlantic salmon followed by gill-cutting, it was observed on EEGs that one out of three fish recovered (Lambooij et al., 2010).

A problem of electrical stunning, especially when fish are immersed in water during stunning, is that carcass damage might occur, such as muscle hemorrhages or a broken vertebral column. Roth et al. (2009) found that this problem could be overcome by exposing fish to the electricity after draining the water, so called 'dry stunning.' In this method, the fish are exposed to an electrical current via a series of rows of positive plate electrodes and a conveyor belt acting as the negative electrode. Evidence shows a positive effect on the quality with a very low incidence of injuries in Atlantic salmon.

Stunning and killing salmonids

Stunning of Atlantic salmon by transferring them into carbon dioxide saturated water has been banned in Norway (Chilvers 2014) and is encouraged to be banned in other countries as well, e.g. Germany (Kleingeld 2013). CO_2 was previously used prior to gill cut, but the method resulted in many fish struggling in panic and many fish never became unconscious.

In Norway, live chilling of Atlantic salmon with controlled addition of low to moderate levels carbon dioxide (65 – 257 mg l^{-1}) and oxygen has been widely used to stun the fish (Erikson, 2006). This method, however, has not yet been assessed with EEG and ECG recordings.

The use of carbon monoxide as a stunning method has so far not been used commercially, but only in research. The method sedates the fish in 5 - 10 minutes and is not immediate (Bjørlykke et al., 2011)

Isoeugenol causes immediate unconsciousness in salmon and is a humane stunning method. However, the chemical is not allowed to use in fish for consumption because of its smell and long retention time (Erikson 2011). However when killing parr or smolt the use of isoeugenol is preferred.

Since trout are too small to be conveniently stunned by percussion, they are traditionally placed directly in ice to die by asphyxia and cooling. In the UK, however, many freshwater trout are electrically stunned before being placed directly in ice where they die by asphyxia before recovery (Lines and Spence, 2012).

Percussive stunning is done by giving a fish a blow to the head with a wooden or plastic club or by using an instrument. One effective blow can disrupt the brain sufficiently to render the fish unconsious and insensible immediately without recovery. Lambooij et al., (2010) showed that when sufficient air pressure is used to drive the bolt in the instrument that is used for percussive stunning, this may lead to carcass damage of Atlantic salmon. Lowering the air pressure can avoid this damage, however, under these conditions percussive stunning is not instantaneous.

For both percussive and electrical stunning applies that when properly done they are considered to be humane (Kleingeld 2013) and without suffer for the fish, and unconsciousness is both immediate and long lasting (Lambooij et al., 2010; van de Vis et al., 2003, Terlouw et al., 2008, Roth et al., 2012). Electrical stunning is applied in practice on Atlantic salmon and trout. The percussive stunning method is effective when the fish are oriented correctly to the stunner. If the fish are not oriented correctly, the hit is either incorrectly placed and causes damages, and/or the fish wakes up following the need for repeated manually strokes in order to kill the fish properly. In a study by Roth et al. (2007), they demonstrated that a force of >75 N with a flat cylinder was required to ensure immediate unconsciousness, that also caused death. Another study by Lambooij et al. (2010) calculated a high probability of effective stun of fish (1,500 g) at 8.1 – 10 bars.

Electrical stunning may be done both in water and out of water. Correct voltage, current or electrical field depends on species, orientation of the fish and also conductivity of the water, duration of exposure, electrical field strength and frequency. Recommended amperages to achieve an instantaneous stun in Atlantic salmon are in Robb and Roth, (2003), Roth et al. (2003, 2004), Lambooij et al. (2010).

Slaughtering crustaceans and shellfish

There is no available literature on bivalves slaughtering, only papers comparing two slaughter processing methods in order to get the best product quality (Fu et al., 2014). At present our knowledge on the central nervous system in shellfish and crustaceans is very limited. Therefore, it is not possible to draw firm conclusions whether or not these animals are able perceive pain (Sneddon et al., 2014).

For crabs and lobsters a limited number of studies on electrical stunning have been performed. However, more studies are needed to conclude whether or not consciousnes and sensibility are lost immediately in these animals by applying electricity (Van de Vis et al., 2015).

6.3 Conclusions and knowledge gaps

When properly done, the most humane stunning methods is percussive and electric stunning. However, percussive stunning may lead to carcass damage, which poses an economical problem. Carcass damage can be avoided by lowering the air pressure in the percussive stunner. However, under the latter conditions it is doubtful that an fish are stunned immediately by percussion.

In case waiting cages are in use, monitoring water quality both with and without crowding should be done. Adding of oxygen when needed.

Pumping should be done with care. Moreover, pumps should be used that were constructed especially for live fish. Make sure to use the correct pump dimensions for the actual fish size and amount. Make sure that the equipment is regularly checked by service

Realistic alternative methods to the ice slurry for stunning and killing marine fish needs to be further investigated. Electrical stunning has to be followed by the application of killing method. However, experiments have shown that fish may recover. To prevent this, further studies are needed to develop protocols for stunning and killing that result in an immediate and irrecoverable stun in fish.

7. State of the art on husbandry - Veterinary treatment

7.1. Current regulation

According to Reg. EC 889/2008, recital 16: "*Animal-health management should mainly be based on prevention of disease. In addition specific cleaning and disinfection measures should be applied*".

Article 25t say:

"*1. When despite preventive measures to ensure animal health, according to Article 15(1)(f)(i) of Regulation (EC) No 834/2007, a health problem arises, veterinary treatments may be used in the following order of preference: (a) substances from plants, animals or*

minerals in a homoeopathic dilution; (b) plants and their extracts not having anaesthetic effects, and (c) substances such as: trace elements, metals, natural immunostimulants or authorised probiotics.
2. The use of allopathic treatments is limited to two courses of treatment per year, with the exception of vaccinations and compulsory eradication schemes. However, in the cases of a production cycle of less than a year a limit of one allopathic treatment applies. If the mentioned limits for allopathic treatments are exceeded the concerned aquaculture animals cannot be sold as organic products.
3. The use of parasite treatments, not including compulsory control schemes operated by Member States, shall be limited to twice per year or once per year where the production cycle is less than 18 months.
4. The withdrawal period for allopathic veterinary treatments and parasite treatments according to paragraph 3 including treatments under compulsory control and eradication schemes shall be twice the legal withdrawal period as referred to in Article 11 of Directive 2001/82/EC or in a case in which this period in not specified 48 hours.
5. Whenever veterinary medicinal products are used, such use is to be declared to the control body or the control authority before the animals are marketed as organic. Treated stock shall be clearly identifiable".
Article 25s(6) say:
"*For biological control of ectoparasites preference shall be given to the use of cleaner fish*".

7.2. Current scientific knowledge

The use of antibiotics in aquaculture has led to the development of antibiotic-resistant bacteria and the accumulation of antibiotics in the environment, resulting in water and soil pollution. Thus, vaccination is the most effective and environmentally-friendly approach to combat diseases in aquaculture to manage fish health.
The use of plants for vaccine production offers several advantages such as low cost, safety and easy scaling. To date a large number of plant-derived vaccines, antibodies and therapeutic proteins have been produced for human health, of which a few have been made commercially available. However, the development of animal vaccines in plants, especially fish vaccines by genetic engineering, has not yet been addressed (Clarke et al., 2006).
The use of plants for development and production of recombinant vaccines offers several advantages. Plant-based systems are more economical as plants can be grown on a larger scale than in other systems. The utilization of plants for low cost and production of large quantities of fish vaccines with oral immunization by plant genetic engineering, especially plastid genetic engineering of edible crops, should be emphasized (Clarke et al., 2013).
The model vaccines include fusion proteins consisting of a gut adhesion molecule (LTB) and a viral peptide or green fluorescent protein (GFP) expressed in potato tubers. The adhesion molecule mediates binding to and uptake from the gut, whereas the viral peptide or GFP functions as model vaccine antigen provoking the induction of an immune response. The authors demonstrate that fusion to LTB facilitates an elevated uptake of the model vaccines in carp gut mucosa. The data presented show the promising potentials of the plant as a production system for oral vaccines in aquaculture and feed mediated immunization. This

report shows promising results for the utilisation of plant-derived cost effective oral vaccines in aquaculture and its application through feed delivery (Companjen et al., 2006).

Natural plant products present a viable alternative to antibiotics and other banned drugs, being safer for the reared organism and humans, as well as the environment (Sagiv et al., 2011).

Recently, increasing attention is being paid to the use of plant products for disease control in aquaculture as an alternative to chemical treatments. Plant products have been reported to stimulate appetite and promote weight gain, stress resistance boosters, to act as immunostimulant and to have antibacterial and anti-parasitic (virus, protozoans, monogeneans) properties in fish and shellfish aquaculture due to active molecules such as alkaloids, terpenoids, saponins, flavonoids, phenolics, polysaccharides and proteoglycans.

The use of medicinal plants in aquaculture has attracted a lot of attention globally and has become a subject of active scientific investigations (Bulfon et al., 2014). The most investigated herbs are those widely used in folk medicine in China, India, Thailand and Korea, such as *Achyranthes aspera, Angelica sinensis, Astragalus membranaceus, Azadirachta indica, Cynodon dactylon, Echinacea purpurea, Massa medicata, Punica granatum, Solanum nigrum, Whitania somnifera, Zataria multiflora*.

Other plants are used all over the world for both curative and culinary purposes, such as garlic, green tea, cinnamon, turmeric, lupine, mango, peppermint, nutmeg, basil, oregano, rhubarb, rosemary and ginger. The herbal remedies consist in plant materials (seeds, bulbs, leaves) or plant-derived products, including extracts obtained using a range of extraction procedures and different aqueous or organic solvents (ethanol, methanol, ethyl acetate, hexane, butane, acetone, benzene, petroleum ether, etc.), or other preparations such as essential oils, concoctions and decoctions (Bulfon et al., 2013).

Herbs such as *S. trilobatum, A. paniculata* and *P. corylifolia* were found to reduce Vibrio in *P. monodon* three time when supplied in enriched Artemia.

Several plant products found to have potent antiviral activity against fish and shrimp viruses.

Antifungal properties were also found in many plants. Herbal compounds have the ability to inhibit the generation of oxygen anions and scavenge free radical, hence reducing stress effects. Other herbs, such as *Astragalus membranaceus, Portulaca oleracea, Flavescent ophora* and *A. paniculata* are known to have specific and none specific anti stress affects.

Nowadays, only a few commercial herbal products are available at a global level for large-scale use in aquaculture. In many countries a review of the current legislation should be undertaken to allow a greater flexibility in their use taking into consideration the benefits that they might have in intensive farming conditions, in terms of fish welfare and public health. Plants and plant bioactives might be proposed in aquaculture primarily as feed additives or immunostimulants, rather that therapeutics, as the registration of herbal remedies to be used in this field is a time-consuming process and implies higher economic costs (Chiara et al., 2013).

Previously, studies have indicated that ginger and/or garlic are effective for the control of a range of bacterial, fungal and parasitic conditions. Also, ginger and/or garlic have been reported to have anti-inflammatory and anti-oxidative activity and to be effective as an immuno-modulatory agents in animals, including fish. Ginger and garlic have been examined for their potential to control *Aeromonas hydrophila* infection in rainbow trout. Ginger and garlic conferred health benefits in terms of a reduction in mortalities after challenge and a heightened effect on non-specific immune mechanisms. Ginger and garlic are recognized to have broad-spectrum activity including activation of phagocytic cells, which is an important component of the non-specific immune system of fish.

The results of those studies reinforce the growing view that some plants are beneficial to fish by conferring protection against disease and stimulating the immune response (Nya and Austin, 2009).

A separate study suggests that salinomycin with amprolium may be a promising treatment for myxosporean infections in intensively cultured warm-water fish, exhibiting action partially via the enhancement of host, innate immune functions and leading to parasite elimination (Karagouni et al., 2005).

Neem (*Azadirachta indica*) is effective and qualifies as a safe and efficient in the prevention of ichthyophonosis in fish. Based on aforementioned results, the following conclusions could be recommended as the effective role of neem in the treatment of Ichthyophonosis in *O. niloticus* fish, since neem stimulated both humoral and cell mediate immunity, and succeeded for the first time to eradicate all the *Ichthyophonus* spores in fish after three months of treatments (Abd El-Ghany et al., 2008).

Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. Antibacterial activity of methanolic extracts from 20 species of macroalgae (9 Chlorophyta, 3 Phaeophyta and 8 Rhodophyta) was evaluated against *Escherichia coli, Staphylococcus aureus* and *Enterococcus faecalis* (Zbakh et al., 2012).

The extracts of the studied 26 marine Rhodophyceae (8 Ceramiales, 7 Gelidiales, 9 Gigartinales, 1 Bonnemaisoniales and 1 Rhodymeniales) (Bouhlal et al., 2010) inhibited considerably the growth of the three tested bacterial strains and gave inhibition zones between 20 and 24 mm. *Staphylococcus aureus* was the most susceptible microorganism (10-35 mm of inhibition). The results indicate that these species of seaweed present a significant capacity of antibacterial activities, which makes them interesting for screening for natural products (Zbakh et al., 2012; Bouhlal et al., 2010).

Immunostimulants such as glucan, chitin, lactoferrin, levamisole, and some medicinal plant extracts or products have been used to control fish and shellfish diseases. The immunostimulants mainly facilitate the function of phagocytic cells, increase their bactericidal activities, and stimulate the natural killer cells, complement, lysozyme activity, and antibody responses in fish and shellfish which confer enhanced protection from infectious diseases.

Administration of herbal extracts or their products at various concentrations through oral (diet) or injection route enhance the innate and adaptive immune response of different freshwater and marine fish and shellfish against bacterial, viral, and parasitic diseases (Harikrishnan et al., 2011).

The development of non-antibiotic and environmentally friendly agents is one of the key factors for health management in aquaculture.

Consequently, with the emerging need for environmentally friendly aquaculture, the use of alternatives to antibiotic growth promoters in fish nutrition is now widely accepted. In recent years, probiotics have taken centre stage and are of use as an unconventional approach that has numerous beneficial effects in fish and shellfish culture: improved activity of gastrointestinal microbiota and enhanced immune status, disease resistance, survival, feed utilization and growth performance. As natural products, probiotics have much potential to increase the efficiency and sustainability of aquaculture production.

The concept of biological disease control, particularly using microbiological modulators for disease prevention, has received widespread attention. A bacterial supplement of a single or mixed culture of selected non-pathogenic bacterial strains is termed probiotics. Probiotics thus are opening a new era in the health management strategy from human to aquatic species including fish and shellfish.

Probiotics were found to stimulate the feed conversion efficiency, augment live weight gain in fish and shrimp culture and confer protection against pathogens by competitive exclusion for adhesion sites, production of organic acids, hydrogen peroxide, antibiotics, bacteriocins, siderophores and lysozyme and also modulate physiological and immunological responses in fish. Moreover, probiotics are also being use as biological control agents in highly stocked intensive aquaculture ponds (Bidhan et al., 2014; Martinez Cruz et al., 2012; Lazado et al., 2014). A wide range of microalgae (*Tetraselmis*), yeast (*Debaryomyces, Phaffia* and *Saccharomyces*), gram-positive (*Bacillus, Lactococcus, Micrococcus, Carnobacterium, Enterococcus, Lactobacillus, Streptococcus, Weissella*) and gram-negative bacteria (*Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas* and *Vibrio*) has been evaluated as probiotics.

Several microalgae, yeasts and gram-positive and–negative bacteria have been isolated from the aquatic medium. Likewise, probiotics have been characterize as new eco-friendly alternative measures of disease control in aquaculture. Generally, probiotics have proven their promising growth results in fish by enhancing the feed conversion efficiency, as well as conferring protection against harmful bacteria by competitive exclusion, production of organic acids, hydrogen peroxide and several other compounds (Bidhan et al., 2014).

In various experiments, probiotics administered to tilapia (*O. niloticus*), increased nonspecific immune response, determined by parameters such as lysozyme activity, neutrophile migration, and bactericidal activity, which improved the resistance of fish to infection by *Edwardsiella tarda*. Other researchers isolated a strain of *Carnobacterium sp*. from salmon bowel and administered alive to rainbow trout and Atlantic salmon, demonstrating in vitro antagonism against known fish pathogens: *Aeromonas hydrophila, A. salmonicida, Flavobacterium psychrophilum, Photobacterium damselae*, and Vibrio species. There is also evidence on the effect of dead probiotic cultures consisting on a mixture of *Vibrio fluvialis* A3-47S, *Aeromonas hydrophila* A3-51, and *Carnobacterium* BA211, in the control of furunculosis in rainbow trout.

For shrimp, studies have focused on the evaluation of probiotics such as *Bacillus cereus, Paenibacillus polymyxa,* and *Pseudomonas sp*. PS-102 as biocontrol agents against pathogens of various Vibrio species. Probiotic strains isolated from the gastrointestinal tract of clownfish (*Amphiprion percula*) have been used to inactivate several pathogens such as *Aeromonas hydrophila* and *Vibrio alginolyticus* among others. Probiotic promote the development of healthy microbiota in the gastrointestinal tract of ornamental fishes from the genera *Poecilia* and *Xiphophorus*, decreasing the amount of heterotrophic microorganisms. It was reported that the use of *Vibrio alginolyticus* strains as probiotics to increase survival and growth of white shrimp, also by using probiotics in Ecuadorian shrimp hatcheries, production increased by 35%, while with the use of antimicrobials it decreased by 94% (Cruz, et al., 2012; Burbank et al., 2011).

Vaccination plays an important role in large-scale commercial fish farming and has been a key reason for the success of salmon cultivation. In addition to salmon and trout, commercial vaccines are available for channel catfish, European sea bass and sea bream, Japanese amberjack or yellowtail (*Seriola quinqueradiata*), tilapia and Atlantic cod (*Gadus morhua*).

In general, empirically developed vaccines based on inactivated bacterial pathogens have proven to be very efficacious in fish. Fewer commercially available viral vaccines and no parasite vaccines exist. Substantial efficacy data are available for new fish vaccines and advanced technology has been implemented. However, before such vaccines can be successfully commercialized, several hurdles have to be overcome regarding the production of cheap but effective antigens and adjuvants, while bearing in mind environmental and associated regulatory concerns (Sommerset et al., 2005).

Veterinary issues in carp pond culture

The health of fish in ponds depends mainly on the environmental conditions and the skills of those who maintain them. Accordingly, the water quality, feeding and the intensity of production are the most important factors which determine the actual health condition of pond fish. Fish should have an overall healthy look and they should search actively for food, react vividly to the received stimuli and disappear quickly if disturbed. Sick fish can be recognized mainly by their behaviour and the condition of their body. Sick fish usually lose appetite, may swim vaguely, stagger, whirl or float. They may concentrate near by the inflowing fresh water or gulp air. They are often thin, covered with wounds and patches, and the body covering mucus can be lost or extremely thick.

Uncontrolled, unregulated and illegal movements of fish unnecessarily increase the risk of spreading fish diseases (Arthur et al., 2004). Before moving live fish of any age groups, strict and professional checking of health should be completed regardless whether it is done between fish farms of a country or countries or even between continents. Fish health examination and its proper certification are not only an important part of transporting fish, but also the most worthy preventions against huge economic losses, which fish diseases may cause. The most important diseases of carp pond fish are:

Spring viremia of carp (SVC) – its pathogen is *Rhabdovirus carpio* (RNS virus). The pathology and clinical signs are changed behaviour, reduced respiratory rate and loss of balance. Uncoordinated swimming and abdominal distension, as well as skin darkening and paleness of gills, are typical symptoms. The most susceptible species are the bighead carp, crucian carp, grass carp, silver carp, common carp, koi carp, European catfish and the tench.

Koi herpes virus (KHV) is caused by a DNA virus. It appears when the water temperature is higher than 22°C. In case of infection, the mortality may be as much as 80 to 100 percent. Its most obvious symptoms are the necrotic white-spotted bleeding gills, sunken eyes, and pale patches and small blisters on the body. These symptoms are often accompanied with secondary bacterial infections. Mainly common and koi carps are infected; however, a majority of Cyprinidae are carriers (Woynarovich et al., 2010).

Vaccination against viral diseases is economically not feasible in carp pond polyculture.

Bacterial diseases

These are responsible for high mortalities in carp fish pond species. The bacteria are usually secondary pathogens. They invade the tissues of the host fish and cause infection. Stress factors and other diseases, such as parasites, make the fish more susceptible to bacterial infections. Water with a high organic load helps the multiplication of bacteria. They cause typically septicaemic and ulcerative symptoms such as scattered haemorrhages and ulcers on the skin, petechial haemorrhages on the gills and on the peritoneum, abdominal swelling and scale losses. In chronic cases, focal lesions may occur in the kidneys and in the spleen as well as in the liver.

The majority of bacterial fish pathogens are Gram negative. The most frequent bacterial fish pathogens are the *Flexibacter*, *Edwardsiella*, *Yersinia*, *Pseudomonas*, *Aeromonas*, *Flavobacterium* and *Mycobacterium*.

For controlling bacterial diseases, different antibiotic therapies can be applied. There are a wide range of treatments against fungus and the different parasites. In order to overcome health problems of fish, aid of a specialized veterinarian is needed, who knows both the diseases and the suitable (efficient, feasible and permitted) treatments. However, the use of antibiotic treatments is not in a good accordance with the principles of organic farming. Hence, a continuous effort is given to the research of various probiotics (e.g., Suantika et al., 2013, Ljubojevic et al., 2013, Xu et al., 2014), immunostimulants (e.g., Pratheepa, Sukumaran 2014, Pionnier et al., 2014, Vaseeharan and Thaya, 2014) and herbal products (e.g., Abasali and Mohamad, 2010, Harikrishnan et al., 2011, Babahydari et al., 2014) which are compatible with the principles of carp organic culture.

Fungal infectious agents, which frequently appear in carp polyculture, are *Saprolegnia* which develops very quickly on dying or dead tissue, readily on lesions and appears in white patches which show its obvious presence. *Branchiomyces* infection is the fungal infection of the gills, which cause necrosis of the tissues in severe cases. Infection may be diagnosed by finding the hyphae and the spores of the fungus in fresh preparations from gills. The fungus attacks the lumen of the blood vessels of the gills and causes blockage, haemostasis, thromboses and necrosis of the affected gill filaments.

Parasites

Protozoans

The most frequent protozoan parasites are flagellates, ciliattes ans sporozoan pathogens (*Ichthyobodo necator*, *Ichthyophthirius multifiliis*, *Trichodina* sp., *Chilodonella* sp., Myxosporea), which live on the surface of body and gills. They spread from fish to fish, but they die quickly in water without host fish. They can infect all fish species.

Platyheminthes

They can be monogeneas (*Gyrodactylus*, *Dactylogyrus*), digeneas (*Sanguinicola*, *Diplostomum*) and cestodes or tapeworms (*Khawia*, *Bothriocephalus*). Almost all of the monogeneas are ectoparasites on the skin, gills and fins of fish where they are hooked on the tissue and they dissolve it with their enzymes in order to make it consumable for them. Cestodes live on the mucous membrane of the intestines from where they suck the already digested food.

Crustaceans

Argulus, *Ergasilus* and *Lernaea* are the most frequent crustacean parasites (ectoparasites) of fish. They live and feed on the skin of fish.

The treatments of parasitic diseases are not feasible in carp pond polyculture due to extremely high water volumes which should be necessary to treat.

Environment and management-related diseases may develop because of stress factors which make fish susceptible to clinical diseases. This happens after thermal shock, overcrowding and traumas occurring during handling and transportation. Irregular or inadequate feeding can also stress fish. The efficient measure for keeping fish healthy is through prevention, which includes the maintenance of a proper rearing environment, ensuring adequate feeding and fish friendly handling.

Shellfish

The class Bivalvia is formed by laterally compressed animals and with the soft body completely or partially enclosed by the shell, which is composed of two hinged valves. The gills are well developed organs, specialized not only for respiration but also for filter-feeding. (Helm et al., 2004). The main bivalve species used in marine aquaculture belong to the families Mytilidae (genus *Mytilus* and *Perna*), Ostreidae (genus *Crassostrea*, *Ostrea* and *Saccostrea*), Veneridae (genus *Ruditapes* and *Mercenaria*) and Pectinidae (genus *Patinopecten*, *Argopecten* and *Pecten*), i.e., mussels, oysters, clams and scallops (FAO). Diseases of mollusks can have a bacterial, viral or parasitical origin, but in shellfish farming, there are no antimicrobial agents available for treatment of molluscan diseases. The culture of bivalves in the hatchery is frequently affected by serious disease outbreaks, mainly related to bacterial infections caused by members of genus *Vibrio*. The disease outbreaks caused by bacterial pathogens can compromise the regular production and the economic viability of the industry (Prado et al., 2010). There are many studies about these outbreaks, but only a few focus on the control of microbiota. The particularities of bivalve aquaculture in a hatchery must be taken into account to design methods of control. A common environment is shared by larvae and bacteria, including both beneficial and potentially pathogenic species. The filter-feeding behavior of larvae increases the strong influence of bacteria on bivalves.

Despite the numerous descriptions of outbreaks in hatcheries, the scarcity of systematic and indeep studies and therefore the lack of scientific knowledge about bacterial populations associated with these cultures have led to the search of partial solutions, wich mostly focused on the elimination of the microbiota from the culture seawater. The different methods employed until present, from treatment of the rearing water to chemotherapy, have shown limited success in avoiding mortalities (Prado et al., 2010).

The majority of diseases affecting mollusks are viral or parasitical and it is not viable to use any antimicrobial agents in open water culture. In addition, the parasitical diseases of shellfish are often intracellular (e.g. *Bonamia ostreae*) and there are no treatments available. It may be possible to observe behavioral changes in some stocks, particularly broodstock and larvae in the hatcheries, and detailed documentation of farm management (e.g. water quality parameters) may also be helpful to manipulate local environmental conditions. However, this is only feasible under controlled hatchery conditions, since diseases can break out very quickly in susceptible stocks. Feeding behavior of larval stages may also give early indications of health problems. Signs of weakening (e.g. gaping shells) in juvenile or adult stages can also be used to predict potential problems, as can decreased movement in motile species (e.g. scallops, clams) (Rodgers and Furones, 2009).

Specific alternative control measures are therefore required for mollusks. These generally include reduced stocking density, altered salinities and lower water temperatures, and preventive measures to introduce or prohibited the transfer of shellfish from known zootic areas. The development of resistant stocks of oysters, particularly for the potential control of *Perkinsus spp*. or *Haplosporidium spp*., has also been suggested, but the possibility of creating sub-clinical carriers of the pathogens could be an additional problem with such stocks (Rodgers and Furones, 2009). The use of probiotics loose in the water might be a good approach to fight against bacterial diseases. At the moment, however, there are very few studies and in general the results of these studies don't show the desired effect. Furthermore these studies are not targeting viral and parasitical diseases, which cause the highest mortality in bivalves.

Homeopathy in fish

At present there is no or very limited information on documented positive effects of homeopathic treatments in fish. There are some papers about the use of homeopatila in tilapia in Brazil, mainly focused on meat quality. Some people are also trying to use homeopathic medicine to treat ornamental fish, mainly Koi carp, guppies, and recently, some homeopathic medicine have been authorized from Italian Ministry of Health - *Arnica Compositum Veterinario; Belladonna Homaccord Veterinario; Berberis Homaccord Veterinario; Atropinum Compositum Veterinario; Echinacea Compositum Veterinario; Discus Compositum QP Veterinario; Nux Vomica Homaccord Veterinario e Zeel Veterinario* (GU n° 12 del 16/01/2015 and GU n° 13 del 17/01/2015) to be used in animals, fish included, but no information about fish diseases and their treatment are available.

7.3. Conclusion and research gaps

Antibiotic use is an integral part of conventional intensive animal agriculture and aquaculture. Increased public concern about antibiotic resistance and the need to preserve the ever-diminishing arsenal of antimicrobials that work in humans for as long as possible, has brought about increased scrutiny of the use of antibiotics – especially for prophylactic and growth enhancing purposes. In accordance with European regulations and to limit the phenomenon of antibiotic resistance, studies are being implemented on the use of herbal or homeopathic medicine and probiotics, which are administered in addition to the feed (Prein et al., 2012).

At the moment Europe is considering a revision of the European regulation relating to veterinary medicinal products (proposal for a regulation of the European parliament and of the council)-COM (2014) 558 final. In this proposal article 4 defines “Veterinary medicinal product” as any substance or combination of substances which fulfils at least one of the following conditions: (a) it is presented as having properties for treating or preventing disease in animals; (b) its purpose is to be used in or administered to animals with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis; (c) its purpose is to be used for euthanasia of animals.

"Substance" means any matter of the following origin: (a) human, (b) animal, (c) vegetable, (d) chemical.
"Homeopathic veterinary medicinal product" means a veterinary medicinal product prepared from homeopathic stocks in accordance with a homeopathic manufacturing procedure described by the European Pharmacopoeia or, in the absence thereof, by the pharmacopoeias used officially in Member States.

In recent years is increasing experimental evidence and studies of probiotics and herbal medicine, and the first results seem to confirm their effectiveness in the prevention and management of diseases affecting aquatic animals breeding.
The use of these substances is permitted in accordance with article 25(t) of Regulation 889/2008, but does not describe in what way and in what quantities are to be administered and they are authorized. It would be appropriate to make a list of such microorganisms and plants which can be used in the composition of the feed, for example, as shown in the register of animal feed additives of the Annex to Regulation 2003/1831 (* extracts and microorganisms).
The extracts of the following plants have been tested to prove their effectiveness against diseases that primarily affect livestock, particularly if they are effective against bacteria, such as *Aeromonas sp., Vibrio sp*., other microorganisms, viruses, fungi and parasites. The plants tested were: *Solanum trilobatum, Andrographis pani culata (*), Psoralea corylifolia, Astragalus membranaceus (*), Portulaca oleracea, Sophora flavescens, Zingiber officinale (*), Allium sativum, Origanum vulgare (*), Azadirachta indica (*)*, marine algae, *Rhodophyceae, Achyranthes aspera, Angelica sinensis (*), Cynodon dactylon, Echinacea purpurea (*), Massa medicated, Punica granatum (*), Solanum nigrum, Whitania somnifera (*), Zataria multiflora*.
The most tested probiotics which have given the best results in the trials were: microalgae (*Tetraselmis*), yeasts (*Debaryomyces, Phaffia, Saccharomyces*), Gram-positive bacteria (*Bacillus* (*), *Lactococcus (*), Micrococcus, Carnobacterium, Enterococcus (*), Pediococcus (*), Lactobacillus (*), Streptococcus (*), Weissella*) and Gram-negative bacteria (*Aeromonas, Alteromonas, Pseudomonas, Vibrio*).
There are initial investigations and tests with regard to the preparation of vaccines derived from the study of genetic engineering, such as DNA vaccines (Regulation 2003/1829 article16), and proteins produced from GMOs. From the first studies we can see how it is possible to produce new solutions for disease prevention obtaining vaccines and immunostimulants low-cost and low environmental impact. It would be interesting to continue to do studies and tests in this direction, since the Regulation 834/2007 article 4 allows for the use of GMOs for Veterinary Medicinal Products.

8. State of the art on husbandry - Biosecurity

8.1. current regulation

According to Reg. EC 889/2008, recital 16: "*Animal-health management should mainly be based on prevention of disease. In addition specific cleaning and disinfection measures should be applied*".

Article 6e: "*1. Bio-fouling organisms shall be removed only by physical means or by hand and where appropriate returned to the sea at a distance from the farm.*
2. Cleaning of equipment and facilities shall be carried out by physical or mechanical measures. Where this is not satisfactory only substances as listed in Annex VII, Section 2 may be used".
Article 25s: "*1. The animal health management plan in conformity with Article 9 of Directive 2006/88/EC shall detail biosecurity and disease prevention practices including a written agreement for health counselling, proportionate to the production unit, with qualified aquaculture animal health services who shall visit the farm at a frequency of not less than once per year and not less than once every two years in the case of bivalve shellfish.*
2. Holding systems, equipment and utensils shall be properly cleaned and disinfected. Only products listed in Annex VII, Sections 2.1 to 2.2 may be used.
3. With regard to fallowing: (a) The competent authority shall determine whether fallowing is necessary and the appropriate duration which shall be applied and documented after each production cycle in open water containment systems at sea. Fallowing is also recommended for other production methods using tanks, fishponds, and cages; (b) it shall not be mandatory for bivalve mollusc cultivation; (c) during fallowing the cage or other structure used for aquaculture animal production is emptied, disinfected and left empty before being used again.
4. Where appropriate, uneaten fish-feed, faeces and dead animals shall be removed promptly to avoid any risk of significant environmental damage as regards water status quality, minimize disease risks, and to avoid attracting insects or rodents.
5. Ultraviolet light and ozone may be used only in hatcheries and nurseries".

8.2. Current scientific knowledge

Biosecurity in aquaculture consists of practices that minimize the risk of introducing an infectious disease and spreading it to the animals at a facility and the risk that diseased animals or infectious agents will leave a facility and spread to other sites and to other susceptible species. These practices also reduce stress to the animals, thus making them less susceptible to disease. Good biosecurity measures will reduce the risk of catastrophic losses from infectious disease and low-level losses that, over time, can also greatly affect the bottom line. A comprehensive biosecurity plan should include the following activities:

- A monitoring system of water quality and fish health.
- Isolating sick fish and removing dead and moribund fish.
- Knowledge which diagnostics to use and which treatments are legal and available.
- Education of personnel and visitors so that they understand and follow biosecurity protocols.
- Keeping good records. Compliance with, and documentation of, biosecurity protocols.
- Always consult product labels for appropriate concentrations, use, shelf life, and safety precautions.
- Consult with the state environmental control agency or the hazardous waste representative at the nearest EPA regional office for guidance on the proper disposal of each product (Oidtmann et al., 2011; Galli et al., 2014; Pietrak et al., 2014; Manual of

Diagnostic Tests for Aquatic Animals OIE., 2009; Southern Regional Aquaculture Centre (SRAC) 2013).

Effective biosecurity plans must be tailored to a specific farm site, be adaptable, address local disease threats, and avoid environmental insult. The biosecurity policies and practices of an aquaculture company are controlled directly by the farmer. The goals of these policies and practices match those of the various levels of government regulated biosecurity, i.e., to reduce the probability that a pathogen will infect one or more animals under the farmer's care or negatively impact the surrounding farms or environment.

A good biosecurity plan, consistently implemented, functions as a type of insurance policy against disease. The routine use of biosecurity measures (secure water supply, healthy fish or shellfish stock, good hygiene practices for all entering and exiting the farm) can reduce the risk of introduction and economic impact of these diseases on the farm (Pietrak et al. 2014).

The washing and disinfection procedures should at least include the following stages:

a) Removal of solid waste, etc., followed by prewashing;
b) Deep cleaning and washing;
c) Disinfection;
d) Rinsing.

The process should be monitored throughout by a technically competent person and records need to be kept (Manual of Diagnostic Tests for Aquatic Animals OIE., 2009).

For carp breeding, when hygienic measures are necessary, quick lime (CaO) is permitted to be applied on to the humid pond bottom (max. 200 kg ha^{-1}). Its application into the pond (max. 150 kg ha^{-1}) for the purposes of pH-stabilisation and for precipitating of suspended organic matter is permitted in critical weather situations (Adámek et al., 2014, Horváth et al., 2015).

For the culture of *Salmonidae, Coregonidae, Gadidae*, sea bream, sea bass and croakers/drum for controlling sea lice in marine net cages, stocking with wrasse as "cleaner fishes" is recommended; for the protection of net cages against growth of algae and colonization by invertebrates, environment-friendly methods shall be employed.

Health status of animals shall be monitored and documented on a regular basis. Special efforts shall be made to detect possible correlations between management measures, manifestation of viral diseases, and reason for mortalities, individual growth and yields/biomass development.

8.3. Conclusion and research gaps

Good hygiene practices and farm management prevent the onset of diseases. Unfortunately, there is currently no European guidelines on biosecurity in animal husbandry, but there are at national level, in the various countries of the EU, for certain species. It would be appropriate in future years draft biosecurity measures recognized at Community level.

9. Ethical considerations as related to welfare, health, veterinary treatment and biosecurity

Fish welfare

Enabling animals to perform natural behaviour, typically also in an environment to which they are biologically adapted (Algers 1992) is often regarded ethically important since animals are sentient. If they were not, natural behaviour would be less decisive for judgement of their welfare, and for legislation (Swedish Animal Welfare Act, SFS 1988:534 4§) or private standards such as KRAV, Debio, Naturland, FairFish etc. Natural behaviour is, in the animal welfare literature, regarded as one of three parameters for measuring welfare, the other two being biological functioning and subjective experiences (Fraser et al., 1997). It is therefore interesting to note a gap between consumer/citizen views on what is important for animal welfare in animal husbandry (Boogaard et al., 2011), and literature on fish welfare in aquaculture. Most of the referred literature in this review relate welfare to biological functioning/health issues e.g. in terms of stress (cortisol) measurement, osmoregulation, fin erosion or body injury, supressed growth rate, elevated metabolic rates, disease and mortality and not to subjective feeling of the fish, whereas a few consider behaviour parameters. This is the case for example in papers on welfare assessment during transport (Nomura et al., 2009) who looked at four behavioural indicators: i) Fish orientation, ii) fish density (nearest neighbor distance, NND was used as a proxy for fish density) iii) swimming effort (tail beat frequency) and iv) overt erratic behaviour (Alänärä and Brännäs 1996).
Although some consumers tend to think that fish is a semi-animal, half a vegetable (Kupsala et al., 2013), some increased interest for fish welfare can be seen in Europe (Huntingford et al., 2006). If an aim with revision of organic regulation would be to widen the gap to regulation of and standards for conventional fish farming, behavioural studies on all farmed species would be most valuable, as well as education and training of employees to safeguard transport and handling practices are not at the cost of fish welfare. If adaptation of farming practices to fish welfare also in terms of behavioural needs would be realized, the suggestion to formulate regulation in relation to freshwater and seawater phases is a constructive step, as well as to establish thresholds and recommendations related to species. It is worth noting though, that this would not be entirely new, but rather to live up to EC Reg. 834/2007, recital (17), (EU, 2007): ”Organic stock farming should respect high animal welfare standards and meet animals' species-specific behavioural needs while animal-health management should be based on disease prevention.” In EC Reg. 889/2008, art. 25f 1/2/3/4/5, (EU, 2008): ”Aquaculture Husbandry practices. General aquaculture husbandry rules” some more detailed instructions are given: d) in the case of freshwater fish the bottom type shall be as close as possible to natural conditions; (e) in the case of carp the bottom shall be natural earth. Hence one is tempted to think that species-specific treatment and procedures related to the different stages of production of freshwater and seawater should already be standard. If this is not the case, why else the suggestion? One is tempted to ask whether this is due to room for different interpretations of legislation, or simply lack of knowledge regarding the legislation among aquaculture farmers and certifiers?

Again, this relates to the overarching, principal, questions a) keep consumers ignorant, and b) whether a large or small discrepancy between organic and conventional practices is to prefer? As to the latter, it is possible to expect that the more clear the difference is, the easier it is for consumers to know and to make an informed choice, of either product. This point was addressed in the stakeholder event in Istanbul October 2014 showing a concern for both lack of consumer knowledge about labels, and lack of difference between organic and conventional. Consumption patterns are however much more complex than based on these two parameters (fish welfare and ideology/values). Among other things, according to the Eurbarometer in 2007, traditions, habits, price, exposure in the store matter.

Given that fish welfare would be considered important by a larger part of society in a near future, but the interest among producers to change systems is low, another issue of principal interest arises. Would it be advisable for organic aquaculture to take the approach of striving to increase fish welfare in total? I.e. based on a utilitarian reasoning organic aquaculture could argue for taking small steps in order to include as many individual fish as possible, i.e. reach most large scale producers. How to take the responsibility of knowledge of fish capacities (mentioned above), IFOAM's principles of naturalness, care and health, and legislator's claim to *pay full regard to the welfare requirements of animals* seriously? Alternatively, this could be interpreted to lead to an approach of respecting each individual fish' welfare, if taking also the formulation of EC Reg. 834/2007, Art. 15 1(b) (vi) re Production rules for aquaculture animals seriously: ”any suffering of the animals including the time of slaughtering shall be kept to a minimum”.

Independently of whether the individual welfare is in focus, or the maximising benefit for the highest number approach is considered the most convincing from an ethical point of view, it is clear that implementation of keeping suffering to a minimum would imply large changes in both many steps of the production systems, including transportation, collection to slaughter and stunning and slaughter.

But, what is keeping suffering at a ’minimum’? Most probable it means one should avoid causing unnecessary suffering. This is not much clearer though – unnecessary for whom and under what circumstances? There are at least six possible definitions of ‘unnecessary’: 1) any suffering, 2) caused by a bad character or bad intentions, 3) all suffering apart from such that is in the interest of the animal, 4) exceeding a certain degree (intensity or durability), 5) necessary suffering equals necessary to fulfil human needs, and finally 6) combination of 3 and 5 i.e. that the suffering of animals can be justified if it is necessary in a ‘utilitarian sense’ (Behdadi 2012). In a context where fish traditionally has been regarded a non-sentient, or ‘low-sentient’ it is probably not far-fetched to think definitions 1, 2 and 3 are regarded too strict, no 4 is possible to consider whereas 5 and 6 would be regarded to mirror realistic expectations in practices related to production (including hatcheries, handling of smolt and transport).

According to results from the Istanbul stakeholder discussion in the OrAqua project, welfare is an important issue for stakeholders, and should be considered on species level, or in species groups. Welfare in terms of behaviour were related to both stocking density, cage design, handling procedures and feed/nutrition expressing a broad welfare definition exceeding physiological health parameters. This is well in line with fish welfare research, but one has to keep in mind that there is most probably less awareness on the species related differences between among ‘normal’ consumers, as they are generally less aware of fish welfare than mammalian welfare (Frewer et al., 2005). Again, the role of labels, transparency and the ideal level of consumer knowledge turn out to be important issues in relation to the level of difference between organic and conventional aquaculture. When aiming for a large difference well-known labels, transparency regarding regulation and high level of consumer knowledge regarding production systems are crucial in order to create and maintain support and understanding for higher prices, whereas aiming for smaller differences would call for the opposite. However, some stakeholders in Istanbul stressed that welfare issues are not important only for organic aquaculture, and that 'nature should not be mimicked' as it might not be the best for the fish. Such a stance differs from much traditional organic philosophy, where nature is seen as a 'guide', not least regarding basis for animal welfare by including respect for species specific behaviours (Lund et al., 2001). It hence seems there are at least two somewhat disparate ideas of how best to define welfare expressed by stakeholders, which calls for a thorough discussion regarding welfare definition as applied to organic aquaculture regulation.

Slaughter

The difficulties in assessment of welfare can be highlighted by the difficulty in assessing unconsciousness at pre-slaughter stunning by looking at the fish. In the review paper by Espmark: "Definition of unconsciousness: … Occasional spasmodic convulsions or gasps can be observed in effectively stunned fish and should not be a cause for concern. Since electricity stimulates the muscles directly, all observations need to be made once the electricity has been removed. (Lines and Spence, 2012)." Although there are reliable methods for slaughtering (electric followed by gill cut and percussive stunning followed by gill cut) not causing welfare impairment if properly performed there are variations in success/efficiency due to e.g. currency and whether the fish flow in correct direction (also other factors such as work load and number of fish pumped?). It can be questioned whether requests for certain levels of training and education should be part of the regulation of organic aquaculture.

10. References

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Chapter 3: PRODUCTION SYSTEMS

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1. Introduction and current regulation

1.1. Production Systems

This document is based upon the review of the scientific literature and the overall information on the different production systems in aquaculture, both conventional and organic. The different topics considered are breeding, hatchery and nursery, phyto-Zoo massive culture, land based and cage systems, Recirculation Aquaculture Systems (RAS), mussel and oyster culture, seaweed culture, and Integrated Multitropic Aquaculture (IMTA).

1.2. Current Regulation

The organic production principles embedded in the Commission Regulation (EC) 834/2007 are mainly based upon a holistic vision of the processes, as it is shown in the following steps of the regulation:

Recital 1: “*Organic production is an overall system of farm management and food production that combines best environmental practices, a high level of biodiversity, the preservation of natural resources, the application of high animal welfare standards and a production method in line with the preference of certain consumers for products produced using natural substances and processes. The organic production method thus plays a dual societal role, where it on the one hand provides for a specific market responding to a consumer demand for organic products, and on the other hand delivers public goods contributing to the protection of the environment and animal welfare, as well as to rural development*”.

Recital 22: “*It is important to maintain consumer confidence in organic products. Exceptions from the requirements applicable to organic production should therefore be strictly limited to cases where the application of exceptional rules is deemed to be justified*”.

Specifically, within the Reg. (EC) 834/2007 it is worth to mention:

Article 11 General farm production rules

The entire agricultural holding shall be managed in compliance with the requirements applicable to organic production. However, in accordance with specific conditions to be laid down in accordance with the procedure referred to in Article 37(2), a holding may be split up into clearly separated units or aquaculture production sites which are not all managed under organic production. As regards animals, different species shall be involved. As regards aquaculture the same species may be involved, provided that there is adequate separation between the production sites. As regards plants, different varieties that can be easily differentiated shall be involved. Where, in accordance with the second subparagraph, not all units of a holding are used for organic production, the operator shall keep the land, animals, and products used for, or produced by, the organic units separate from those used for, or produced by, the non-organic units and keep adequate records to show the separation.

Article 15 Production rules for aquaculture animals

In addition to the general farm production rules laid down in Article 11, the following rules shall apply to aquaculture animal production:

(a) with regard to the origin of the aquaculture animals:

i. *organic aquaculture shall be based on the rearing of young stock originating from organic brood-stock and organic holdings;*

ii. *when young stock from organic brood-stock or holdings are not available, non-organically produced animals may be brought onto a holding under specific conditions;*

(b) with regard to husbandry practices:

ii. *husbandry practices, including feeding, design of installations, stocking densities and water quality shall ensure that the developmental, physiological and behavioural needs of animals are met;*
iii. *husbandry practices shall minimise negative environmental impact from the holding, including the escape of farmed stock;*
iv. *organic animals shall be kept separate from other aquaculture animals;*

(c) with regard to breeding:

i. *artificial induction of polyploidy, artificial hybridisation, cloning and production of monosex strains, except by hand sorting, shall not be used;*
ii. *the appropriate strains shall be chosen;*
iii. *species-specific conditions for brood-stock management, breeding and juvenile production shall be established;*

(g) With regard to cleaning and disinfection, products for cleaning and disinfection in ponds, cages, buildings and installations, shall be used only if they have been authorised for use in organic production under Article 16.

2. Breeding

2.1. Regulation

According to Reg. EC 889/2008,

Article 25d (1) “*Locally grown species shall be used and breeding shall aim to give strains which are more adapted to farming conditions, good health and good utilisation of feed resources. Documentary evidence of their origin and treatment shall be provided for the control body or control authority*.”

Article 25d (2) “*Species shall be chosen which can be farmed without causing significant damage to wild stocks*.”

Article 25e (1) “*For breeding purposes or for improving genetic stock and when organic aquaculture animals are not available, wild caught or non-organic aquaculture animals may be brought into a holding. Such animals shall be kept under organic management for at least three months before they may be used for breeding*.”

Article 25h (1) ”*… Brood-stock shall be handled in a manner to minimize physical damage and stress and under anaesthesia where appropriate …*”

Article 25h (2) ”*The following restrictions shall apply to the use of artificial light: (a) for prolonging natural day-length it shall not exceed a maximum that respects the ethological needs, geographical conditions and general health of farmed animals, this maximum shall not exceed 16 hours per day, except for reproductive purposes; (b) Abrupt changes in light intensity shall be avoided at the changeover time by the use of dimmable lights or background lighting.*”

Article 25i “*The use of hormones and hormone derivates is prohibited*.”

2.2. Current scientific knowledge

According to the regulation (EC, 2008) the production cycle shall be fully organic, i.e. 100 % of the juveniles shall be of organic origin from 1st January 2016. When searching the literature no scientific literature related to breeding and sourcing of juveniles in organic aquaculture was found. As seen from the regulation it is said that breeding shall aim to give strains which are more adapted to farming conditions, good health and good utilisation of feed. When organic aquaculture juvenile animals are not available, non-organic aquaculture juveniles, which have been organically managed at least for the latter two thirds of the duration of the production cycle, can be used.

Salmon and trout

Although there are no official data on the number of certified organic hatcheries in Europe, a few hatcheries (e.g. for trout in Denmark) have recently converted or are in the process of conversion to organic production (www.eurofishmagazine.com, June 3/2013). Therefore, the present production of organic juveniles is likely to be inadequate to supply the growing demand of the organic aquaculture industry.

Apart from the lack of organic juveniles, due to the few hatcheries certified as organic, one of the main difficulties experienced by the sector is the restriction on the movement of live animals between countries and regions based on the “Directive 2006/88/EC on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals”. This Directive established five categories of health status in which countries, zones and compartments have to be classified, and rules to be followed for introducing or dispatching animals among areas with different health status classification.

A second barrier to the movements of seed (or juveniles) among farms is due to the reluctance of farmers to introduce on their farms animals which could be unsuitable for the local (geographical) environment (e.g. genetic or population traits, resistance to different diseases, growth performances, reproductive cycle, behavioural characteristics, etc.).

At present there is no literature on any breeding program specifically targeting organic salmon aquaculture. Usually, roe from brood stock from conventionally salmon are used. It is unlikely that breeding companies will develop a genetic material, which is specifically developed for the organic farmers. It would require a lot of resources and there should be a high demand for organic roe for the breeding companies to be able to run a separate breeding program for organic salmon farmers. Thus, it is highly unlikely that the demand in the regulation of the maximum percentage of non-organic aquaculture juveniles introduced to the farm shall be 50% by 31 December 2013 and 0% by 31 December 2015 can be fulfilled.

In addition, at present it is not clear if the breeding objectives and thus targeted traits are sufficiently different to warrant developing genetic material specifically for organic farmers. Traits like salmon lice and some disease resistance traits are most likely of higher importance for organic farmers compared to conventional salmon farmers, since organic farmers cannot use chemical treatments.

Sea bass and sea bream

Under farming conditions, in Mediterranean countries, sea bass males/females ratio is approximately 3:1. Unfortunately, males grow more slowly than females (Saillant et al., 2001). Sexual maturation, that is responsible of the reductions in growth rates, occurs before reaching marketable size. Although in the European organic aquaculture the use of polyploidy is not allowed, in the conventional aquaculture chromosome set manipulation techniques, including pressure, temperature and chemical shocks, are used to obtain triploid, sterile stocks (Felip et al., 1997). The optimum way to induce triploidy in the sea bass is to generate a thermal shock lasting 10 minutes at 0°C, just 5 minutes after egg fertilization (Felip et al., 1997).

Triplody induction was proved to be less effective in sea bream, that, in contrast to sea bass, is a protandrous hermaphrodite species and matures as males when triploidy is induced. Triploid specimens are sterile and show lower growth performances than diploids (Haffray et al., 2005).

Aquaculture management and selection in sea bass is actually sustained by the development of new genetic tools. These are useful in reducing inbreeding, lowering losses of farmed fish from infections, improving resistance to suboptimal environmental conditions, and accelerating growth rate, improving, in turn, the domestication process of the species.

One of the most effective genetic tools are microsatellites or single sequence repeats (SSRs), DNA sequences that show high levels of intra specific allele polymorphism that are widely distributed within vertebrate genomes (Toth et al. 2000). The microsatellites are used in a wide range of application in aquaculture (Chistiakov et al., 2005), such as the evaluation of ploidy levels and paternal non participation in gynogenetic manipulations, the estimation of consanguinity rate, the identification of wild or domestic strains in brood-stock management programs (Garcia de Leon et al., 1998), and to improve disease resistance and slaughter traits (Parati et al., 2012).

Microsatellites were successfully used in sea bass to reduce in aquaculture the inbreeding rate among sibs, giving an effective tool for the parentage identification to be used in aquaculture breeding programmes (Garcia de Leon et al., 1998) such as to assess parentage in sea bass and sea bream (Brown 2003) breeding programme.

In addition, organic ova and juveniles of rainbow trout (*Onchorhynchis mykiss*) have been available from Danish hatcheries since 2013 and are purchased world-wide (www.eurofishmagazine.com, June 3/2013). Strict hygienic conditions are particularly crucial in organic production of ova and juveniles to avoid diseases as medication of organic trout is only allowed within very strong limits. This also underlines the need of robust stress resilient and immunocompetent organic fry.

Carp and other pond fish

Current practice in carp genetics and breeding is based on the application of two approaches in formation of modern breeding programs: classic breeding methods (selection hybridization, sib-mating) and genome manipulations. E.g., in Czech aquaculture the conservation of genetic resources of major commercial fish species including common carp, basic features of breeding programme, trade with fish breeds, their artificial reproduction as well as performance testing of purebreds and F_1 hybrids, data recording and herd book keeping are treated within the Animal Breeding Act. No. 154/2000 of the Code of Laws, in wording of next amendments.

Breeding programme is mostly based on crossbreeding as it brings quick improvement of growth performance (heterosis effect) in F_1 generation. Crossbreeding of breeds developed for common carp improved survival rate of fry, cold and disease resistance (Flajšhans and Hulata, 2006; Piačková et al., 2013). Following the uniform testing methodology with mathematic model for correction of phenotypic values, the on-farm performance testing of purebreds and/or F_1 hybrids of common carp is currently conducted. Strictly done with control groups of different phenotype, embryos for common carp tests are usually shared by several fish farms. Crossbreeding for hybrid vigour display is usually based upon genetic distance of breeds used (Kohlmann et al., 2005). Tests start with pure-breeding, diallele- or mostly top-crossing of P generation with evaluation of reproductive parameters of spawners and continue, always in triplicate, until market size of the tested progeny groups when concluded by evaluation of the slaughtering value. During on-growing of fish, data on survival and growth (expressed in weight) are regularly collected (Kocour et al., 2008).

For conservation of fish genetic resources, the present „National programme of conservation and utilization of genetic resources of farm- and other animals important for nutrition, agriculture and forestry" has been launched already in 1996 with the goal to keep old, less productive breeds as a part of national heritage and as a source of genes for contemporary breeding (Flajšhans et al., 1999). Nowadays, selected Czech fish farms maintain in total 14 live gene banks of common carp with 11 breeds, as well as 22 genetic resources of other pond species (tench, *Tinca tinca*; wels catfish, *Silurus glanis*; great maraena, *Coregonus lavaretus*; Northern whitefish, *Coregonus peled*; sterlet, *Acipenser ruthenus*, and beluga, *Huso huso*). Apart from the on-farm live gene pools, the cryopreserved sperm bank maintains at present 3 238 insemination doses of 11 common carp breeds, as well as 3 595 insemination doses of 18 genetic resources of the other fish species.

2.3. Conclusion and research gaps

At present it is not clear if the breeding objectives and thus targeted traits are sufficiently different to warrant developing genetic material specifically for organic farmers. Traits like parasites (e.g. salmon lice) and some disease resistance traits are most likely of higher importance for organic farmers, compared to conventional farmers, since organic farmers have strict limitations to the use of chemical treatments.

Considering that for breeding purposes or for improving genetic stock and when organic aquaculture animals are not available, wild caught or non-organic aquaculture animals may be brought into a holding (Reg. 889/2008 art. 25e), conventional brood-stock can be used which has been selected based on specific traits. Indeed, some salmon breeding companies offer genetic material, which has a high resistance to IPN, PD or salmon lice (e.g. salmobreed.no or aquagen.no). This will probably allow to meet the provision of the Reg. 889/2008 art. 25d "*… breeding shall aim to give strains which are more adapted to farming conditions, good health and good utilisation of feed resources*".

Since the broodstock is also required to be of organic origin or should be kept under organic management for at least three months it would probably require the fish farmer to keep a number of organically managed broodstock themselves. It will require a strict management of the broodstock population in order to avoid inbreeding in the next generation.

3. Hatchery and nursery

3.1. Regulation

According to Reg. EC 889/2008,

Article 25c (1) “*The competent authority may permit hatcheries and nurseries to rear both organic and non-organic juveniles in the same holding provided there is clear physical separation between the units and a separate water distribution system exists.*”

Article 25e (2) “*For on-growing purposes and when organic aquaculture juvenile animals are not available non-organic aquaculture juveniles may be brought into a holding. At least the latter two thirds of the duration of the production cycle shall be managed under organic management.*”

Article 25e (3) “*The maximum percentage of non-organic aquaculture juveniles introduced to the farm shall be 80 % by 31 December 2011, 50 % by 31 December 2014 and 0 % by 31 December 2015.*”

Article 25e (4) “*For on-growing purposes the collection of wild aquaculture juveniles is specifically restricted to the following cases: (a) natural influx of fish or crustacean larvae and juveniles when filling ponds, containment systems and enclosures; (b) European glass eel, provided that an approved eel management plan is in place for the location and artificial reproduction of eel remains unsolved; (c) the collection of wild fry of species other than European eel for on-growing in traditional extensive aquaculture farming inside wetlands, such as brackish water ponds, tidal areas and costal lagoons, closed by levees and banks, provided that: (i) the restocking is in line with management measures approved by the relevant authorities in charge of the management of the fish stocks in question to ensure the sustainable exploitation of the species concerned, and (ii) the fish are fed exclusively with feed naturally available in the environment.*”

Article 25g (1) ”*Closed recirculation aquaculture animal production facilities are prohibited, with the exception of hatcheries and nurseries or for the production of species used for organic feed organisms.*”

Article 25g (4) “*Artificial heating or cooling of water shall only be permitted in hatcheries and nurseries. Natural borehole water may be used to heat or cool water at all stages of production.*”

3.2. Current scientific knowledge

Larval rearing is one of the most critical stages for the successful propagation of any species and represents one of the major bottlenecks of the whole aquaculture process. Most fish larvae, particularly the marine ones, are very small (total length of approximately 3-4 mm) at first feeding and thus are sensitive to rearing environment and to feed quality. Furthermore, these small larvae require live plankton for their first feeding and thus hatcheries include facilities for plankton production (both phytoplankton and zooplankton), the actual larval rearing zone and also for weaning and nursery. The majority of the hatcheries have also brood-stock facilities, although in some cases transported eggs are used to initiate a production cycle.

A variety of hatchery techniques are available (Divanach and Kentouri, 1999), all sharing a common characteristics, such as the use of phyto- and zooplankton during the period of larval first feeding. The main classifications are based on the rearing density (intensive, semi-intensive, extensive) and the use of phytoplankton in the water (clear, green, pseudo-green) (Papandroulakis et al., 2002).

Independently from the applied method, there are three distinct phases during larval rearing: (i) egg hatching and autotrophic phase when larvae consume their yolk sac reserves, (ii) heterotrophic phase when larvae are fed on zooplankton, and (iii) the weaning to artificial diets. During these phases larvae complete their transformation to juveniles. Juveniles usually remain in the hatchery, for pre-growing, until reaching 2 - 5 g in weight. In cases where on-growing is performed in open sea conditions, the pre-growing period is extended until individuals reach a weight of 10 - 30 g. During this period several procedures are commonly applied, including grading, vaccination and quality control.

Intensive rearing systems for marine larvae

In intensive hatcheries, larvae are reared at high densities under controlled conditions and success is highly depending on the level of knowledge of the larvae's specific biological needs. Intensive rearing is characterized by high stocking densities, controlled conditions of water quality, light intensity, photo-phase and feeding. The most commonly applied method are (i) the "clear water" technique (Coves and Gasset, 1993; Papandroulakis, 2000), with no use of phytoplankton in the rearing medium, (ii) the "green water" technique that is based on the creation of optimum conditions for endogenous phytoplankton bloom of specific organisms in the larval tanks (Saroglia et al., 1989), and (iii) the so-called "pseudo-green water" technology (Papandroulakis et al., 2002), which is based on the frequent addition of phytoplankton and zooplankton in the larval rearing tanks, where phytoplankton is not produced, nor bloom, but its concentration remains constant by daily addition. The pseudo-green method is applied during the most critical segment of the rearing process, at the beginning of larval rearing (until the 20th to 30th day post hatching), when the larvae are still extremely weak, sensitive to alterations in the rearing environment, easily stressed and difficult to feed. After this period, the "clear water" methodology is applied.

Extensive and semi-intensive rearing systems for marine larvae

In extensive hatcheries, larvae are reared at low densities in large tanks or ponds, under more natural conditions, feeding on endogenous blooms of wild marine zooplankton, but there is no industrial application due to the low productivity. As an intermediate approach between the intensive and extensive method, semi-intensive techniques, like the so called "mesocosm technology" (Divanach and Kentouri 1999), have been developed and are applied for the rearing of several species. The actual form of the mesocosm technology was defined after studying the originally applied models of extensive rearing (Grice and Reeve, 1982; Divanach, 1985; Kentouri, 1985; Lalli, 1990). The most important characteristic of the infrastructure required is the size of the larval tanks which should range between 20 to 60 m^3. The conditions of rearing are independent from any climatic and/or seasonal changes. There is a partial control of the light conditions (intensity and photo-phase) and a minimal control of the temperature. The initial egg density in the mesocosm ranges from 4 to 7 eggs l^{-1}, depending on species, and should never exceed 20 eggs l^{-1}. Tanks are filled with natural seawater filtered mechanically, and wild plankton is thus introduced in the system offering a capacity for endogenous production. Phytoplankton is added daily to maintain the green medium for a period of 2 - 4 weeks after hatching. Exogenously produced enriched rotifers, enriched instar II *Artemia sp*. and artificial diet is added when required. The technology has been successfully used for the mass production of several species (Papandroulakis et al. 2004; Kentouri and Divanach, 1983; Ben Khemis, 1997; Koumoundouros et al., 1999; Papandroulakis et al., 2003; Papandroulakis et al., 2005). The mesocosm methodology results in high survival rates and low percentage of individuals with developmental abnormalities while, in general, larval growth performance is better than in the classical intensive systems. Similar semi intensive methods, like the above described, are also applied in different parts of the world, under different names such as "large volume rearing" (Prestinicola et al., 2013; Dhert et al., 1998) where the size of tanks, the rearing density and the presence of wild plankton are critical factors of the process. Recent studies (Prestinicola et al., 2013) concluded that large volume rearing leads to a significant improvement of the morphological quality (i.e., lowered incidence of severe skeletal anomalies and meristic count variability) of gilthead sea bream juveniles reared under semi-intensive conditions. Furthermore, there is evidence that the rearing conditions during the early life stages do have an impact on the behavioural response of sea bass during on-growing, and the individuals reared with the mesocosm method are more sensitive to human presence, presenting behaviour closer to wild individuals (Papandroulakis et al., 2012).

Larval rearing of fresh water species (percid)

The larval rearing of pikeperch is very similar to that of marine fish larvae due to the size of the individuals at first feeding. The temperature is maintained constant at about 18-19°C throughout the larval rearing phase, and gradually increased up to the time of transfer of juveniles to the on-growing tanks. The optimal temperature during on-growing is around 23-25°C. Initial stocking density usually ranges between 20 and 50 larvae L^{-1}, but fish density must be reduced after the weaning phase. Feeding is based on live preys, similar to marine larvae, i.e. rotifers and *Artemia* nauplii. First feeding is composed of enriched rotifers (either the brackish water species *Brachionus plicatilis* or the freshwater species *B. calyciflorus*) or of small size *Artemia* nauplii (350 - 380 µm) for a period of 3 days. Afterwards, larvae are fed enriched *Artemia* nauplii (420 - 450 µm) (Lund, pers. comm.). At 25-30 days after hatching (body weight of 50 - 60 mg), the pikeperch are gradually weaned to appropriate dry feed, by replacing progressively the live prey with a high quality compound feed (300 - 500 µm) within 4 - 5 days.

Larval rearing of carp

Common carp are mainly omnivorous, with animal prey representing more than 75% of the diet. A few days after hatching, the fish larvae feed mainly on small zooplankton, such as rotifers (not enriched) and copepod nauplii. After a short period, however, they shift to larger organisms such as cladocerans and copepods (Dulic et al., 2011; Nunn et al., 2012) or, seldom, to non-enriched *Artemia nauplii*. This change occurs gradually, largely depending on the size of the fish mouth, which is also correlated with body size. The size at which individuals shift from planktivorous to benthivorous feeding habit, however, depends on many factors, such as the availability of planktonic and benthic food, as well as the ratio between both types of food. Crustaceans will form the main component of the feed until individuals reach 100 - 150 mm. The amount of zooplankton ingested increases with fish size. From the juvenile stage onward, carp is primarily a bottom feeder, and aquatic insects (mainly benthic larvae of chironomids) form the main component of the diet.

Microbial control in hatchery

As in any aquaculture operation, microbial control in hatcheries is essential and standard disinfection methods are applied for the facility and the equipment used.

In marine species fish eggs disinfection is not frequently practised. While it is considered mandatory in salmonid species. The World Organisation for Animal Health (OIE) recommends the use of iodophors for egg disinfection (OIE manual, 2012). Hydrogen peroxide is also used for disinfection of both eggs and live prey, before administering the prey to the larvae.

The aquatic environment is more supportive to pathogenic bacteria, independently of their host, than the terrestrial environment and, consequently, pathogens can reach high densities around the animals, which then ingest them either with the feed or when they are drinking. As a consequence, culturing several species of aquatic animals (especially larval stages) in many cases suffers from highly unpredictable survival rates because of bacterial diseases (Verschuere et al., 2000). Independently from the species reared, no antibiotics are generally used in larval rearing, as larvae are in general very vulnerable and might not tolerate the treatment. Hence, techniques to control pathogenic bacteria are paramount to the further development of the aquaculture sector.

In the review of Defoirdt et al. (2007) a critical evaluation is presented of alternative measures that have recently been developed to control disease caused by *Vibrio harveyi* and closely related bacteria. Techniques discussed include phage therapy, the use of short-chain fatty acids (SCFAs) and *polyhydroxyalkanoates*, quorum-sensing disruption, probiotics and “green water”. Some of the techniques have only been studied recently and have only been tested in the laboratory (e.g. disruption of cell-to-cell communication), whereas others have a longer history, including farm trials (e.g. the application of probiotics). Each of the techniques has its advantages but also its limitations. In fact, none of them will probably be successful in all cases. Therefore, it is of importance to develop further all of these alternatives to construct a toolbox containing different sustainable measures. A good management strategy might then use different techniques in rotation to prevent resistance development. Alternatively, it might be valuable to determine which techniques are, and which are not, compatible with each other, to apply them together to maximize the chance of protecting the animals successfully.

Weaning procedure

Feed mixes for weaning are different from those for on-growing. Their production in organic quality is technically possible, therefore, feed manufacturers might be interested to produce such feeds, if there is sufficient demand.

3.3. Conclusion and research gaps

There are no official data on the number of certified organic hatcheries in Europe, except for some information on a few trout hatcheries in Denmark that have recently converted or are in the process of conversion to organic production.

Therefore, the present production of organic juveniles seems inadequate to supply the growing demand of the organic aquaculture industry certified according to the European regulation.

There is a lack in the Regulation of specific organic rules for managing the life cycle stage between the hatching and the weaning of juveniles. This lack of organic regulation concerns fresh water species (e.g. stocking density, feeding) and, even more, marine species (e.g. phytoplankton and zooplankton production, essential nutrients in the trophic chain, feeding, stocking density during larval rearing and weaning, husbandry environment).

Production rules for the phase of the life stage between hatching and weaning of juveniles would have a strong influence in determining the characteristics of the adult (e.g. skeletal and pigmentation anomalies, immune resistance, etc.).

For marine fish, there is evidence that juveniles produced with “mesocosm” or “large volume rearing” systems are more similar in behaviour and morphology to their wild counterparts.

An outstanding lack of amendment to the regulation/knowledge gap is the lack of specific organic rules for managing the life cycle stage between the hatching and the weaning of juveniles for specific species in fresh water, but particularly marine species. Further, the current regulation is not distinguishing between organic and non-organic hatcheries.

4. Phyto-Zoo massive culture

4.1. Regulation

According to Reg. EC 889/2008,

Article 25a “*This Chapter lays down detailed production rules for species of fish, crustaceans, echinoderms and molluscs as covered by Annex XIIIa. It applies mutatis mutandis to zooplankton, micro-crustaceans, rotifers, worms and other aquatic feed animals.*”

Article 25l (a) “*In the larval rearing of organic juveniles, conventional phytoplankton and zooplankton may be used as feed.*”

Article 25m (1) “*Feed materials of mineral origin may be used in organic aquaculture only if listed in Section 1 of Annex V.*”

Article 25m (2) “*Feed additives, certain products used in animal nutrition and processing aids may be used if listed in Annex VI and the restrictions laid down therein are complied with.*”

4.2. Current scientific knowledge

Mass-production of phytoplankton

Phytoplankton is of major importance in the hatchery process, having a double role. It is used in the rotifer cultures, either as feed or as enriching media, and also as medium for improvement of the rearing environment of larvae. Its role for larval rearing includes antibacterial properties, shading effect that improves larvae predation and trigger effect for feeding behaviour or physiological processes (Scott and Baynes, 1979; Naas et al., 1992; Tamaru et al., 1993; Reitan et al., 1993; Cahu et al., 1998; Van der Meeren 1991).

In all cases, the cultures are started from selected strains followed by an upscale in production (increase in volume) and are based on three operations: (i) strain maintenance, (ii) pre-cultures and (iii) mass cultures. The mass culture is usually performed in plastic bags or more recently in photo-bioreactors at high cell density (Tredici and Materassi, 1992; Pulz 2001). Commercial nutrient solutions needed for mass-production of phytoplankton contain all necessary macro and micronutrients, silicates and vitamins in easily soluble, mineral form

(Vonshak, 1986; Smith et al., 1993; Lavens and Sorgeloos, 1996). Carbon dioxide is regularly supplied for phytoplankton cultures (especially in reactors) as a nutrient source.

Mass-production of zooplankton

Two genera of zooplankton are mass cultured due to their appropriate size and easiness of mass culture. These are (i) the rotifer *Brachionus sp*. and (ii) the nauplius of the branchiopod crustacean, *Artemia sp*. Rotifers are the initial prey for the majority of marine fish larvae and are later replaced by *Artemia sp*. during the larval rearing process. Appropriate methods have been developed also for the culture of some ciliate species and for some copepods (Lavens and Sorgeloos, 1996, Marcus 2005, A. Tandler pers. comm.).

Rotifers are an excellent first feed for fish larvae because of their small size and slow swimming speed, their habit of staying suspended in the water column and their ability to be cultured at high densities due to a high reproductive rate (Dhert et al., 2001). As with microalgae, there are many recognized techniques for culturing rotifers. Production may be extensive in large 50 to 150 m^3 tanks, or intensive in small tanks of 1 to 2 m^3. Culture methods are classified as either batch, semi-continuous, or continuous.

For the feeding of rotifers several products are used, sometimes in combination, such as baker's yeast, different algal species, locally produced or purchased as algal paste, and formulated feeds.

Artemia sp. is collected as dehydrated embryos or cysts from salt lakes and salt works. It is used either as instar I nauplii (400-600 micro-meters) hatched from cysts or as instar II-III nauplii (800-1000 micro-meters), reared with specially enriched feed. Frequently, cysts are de-capsulated with hypochlorite prior to hatching, in order to allow both preliminary disinfection of prey and better hatching rates (Lavens and Sorgeloos, 1996). Recently, other methods are applied which do not require de-capsulation: Artemia cysts are coated with non-toxic ferro-magnetic material (SepArt). After hatching the cysts, the nauplii and unhatched cysts are drained or siphoned into a separator that contains a magnet. Thus, unhatched cysts are trapped by the magnet, while nauplii are ready to use. Hatching and culture is performed in columns with high aeration at temperatures of about 26°C.

Rotifers and Artemia can be enriched in highly unsaturated fatty acids (EPA and DHA) and vitamins (C and A) and this can be done with microalgae (local cultures, algal pastes or powders of *Thraustrochytrids* single cell products), as well as oil emulsions. Commercial products are made up with synthetic antioxidants and emulsifiers, and do not comply with organic standards.

4.3. Conclusion and research gaps

There is a potential conflict with the principles of organic production. In the organic production of terrestrial crops, it is an overall principle that plants must not be fertilized with easily soluble nutrients. Art. 4(b)(iii) of Reg. 834/2007 limits the use of fertilizers to "low solubility mineral fertilizers". In the implementing rules, hydroponic production is prohibited (Art. 4 of Reg. 889/2008). It is clear that this principle was developed for terrestrial plants, and does not hold for aquatic production, i.e. phytoplankton, where the nutrients are only available in soluble form. In the case of vitamins and other substances, the same rules concerning GMO risk should apply as for feed of terrestrial animals.

It seems rather difficult to find characteristics sufficiently different between organic and conventional phytoplankton productions, enough to justify the existence, as a separate product, of organically certified phytoplankton.

However, in view of the necessity to use phytoplankton in hatchery, its use could be authorized without requiring organic certification, with the sole exclusion of GMO strains of algae.

Unlike phytoplankton, there could be the technical possibility of an organic production of zooplankton, which would differ from conventional zooplankton in several aspects. Rules for organic production would need to be based on use of organic yeast, other microorganisms (e.g. *thraustrochytrids*), and only natural antioxidants, vitamins and emulsifiers. Unfortunately, at moment, there are no organic enrichment diets available and an evaluation whether their production would be commercially viable would be very useful to be explored.

5. Land based and cage systems

5.1. Regulation

According to Reg. EC 889/2008,

Article 6b (1) “*Operations shall be situated in locations that are not subject to contamination by products or substances not authorised for organic production, or pollutants that would compromise the organic nature of the products.*”

Article 6b (2) “*Organic and non-organic production units shall be separated adequately. Such separation measures shall be based on the natural situation, separate water distribution systems, distances, the tidal flow, the upstream and the downstream location of the organic production unit. Member State authorities may designate locations or areas which they consider to be unsuitable for organic aquaculture or seaweed harvesting and may also set up minimum separation distances between organic and non-organic production units. Where minimum separation distances are set Member States shall provide this information to operators, other Member States and the Commission.*”

Article 6b (3) “*An environmental assessment proportionate to the production unit shall be required for all new operations applying for organic production and producing more than 20 tonnes of aquaculture products per year to ascertain the conditions of the production unit and its immediate environment and likely effects of its operation. The operator shall provide the environmental assessment to the control body or control authority. The content of the environmental assessment shall be based on Annex IV to Council Directive 85/337/EEC (1). If the unit has already been subject to an equivalent assessment, then its use shall be permitted for this purpose.*”

Article 6b (4) “*The operator shall provide a sustainable management plan proportionate to the production unit for aquaculture and seaweed harvesting. The plan shall be updated annually and shall detail the environmental effects of the operation, the environmental monitoring to be undertaken, and list measures to be taken to minimise negative impacts on the surrounding aquatic and terrestrial environments, including, where applicable, nutrient discharge into the environment per production cycle or per annum. The plan shall record the surveillance and repair of technical equipment.*”

Article 6b (5) *"Aquaculture and seaweed business operators shall by preference use renewable energy sources and re-cycle materials and shall draw up as part of the sustainable management plan a waste reduction schedule to be put in place at the commencement of operations. Where possible, the use of residual heat shall be limited to energy from renewable sources."*
Article 25b (2) *"Defensive and preventive measures taken against predators under Council Directive 92/43/EEC (1) and national rules shall be recorded in the sustainable management plan."*
Article 25b (4) *"For aquaculture animal production in fishponds, tanks or raceways, farms shall be equipped with either natural-filter beds, settlement ponds, biological filters or mechanical filters to collect waste nutrients or use seaweeds and/or animals (bivalves and algae) which contribute to improving the quality of the effluent. Effluent monitoring shall be carried out at regular intervals where appropriate."*
Article 25c (2) *"In case of grow-out production, the competent authority may permit organic and non-organic aquaculture animal production units on the same holding provided Article 6b(2) of this Regulation is complied with and where different production phases and different handling periods of the aquaculture animals are involved."*
Article 25f (1) *"The husbandry environment of the aquaculture animals shall be designed in such a way that, in accordance with their species specific needs, the aquaculture animals shall: (a) have sufficient space for their wellbeing; (b) be kept in water of good quality with sufficient oxygen levels, and (c) be kept in temperature and light conditions in accordance with the requirements of the species and having regard to the geographic location; (d) in the case of freshwater fish the bottom type shall be as close as possible to natural conditions; (e) in the case of carp the bottom shall be natural earth."*
Article 25f (3) *"The design and construction of aquatic containment systems shall provide flow rates and physiochemical parameters that safeguard the animals' health and welfare and provide for their behavioural needs."*
Article 25g (2) *"Rearing units on land shall meet the following conditions: (a) for flow-through systems it shall be possible to monitor and control the flow rate and water quality of both in-flowing and out-flowing water; (b) at least five per cent of the perimeter ('land-water interface') area shall have natural vegetation."*
Article 25g (3) *"Containment systems at sea shall: (a) be located where water flow, depth and water-body exchange rates are adequate to minimize the impact on the seabed and the surrounding water body; (b) shall have suitable cage design, construction and maintenance with regard to their exposure to the operating environment."*
Article 25g (4) *"Artificial heating or cooling of water shall only be permitted in hatcheries and nurseries. Natural borehole water may be used to heat or cool water at all stages of production."*
Article 25h (3) *"Aeration is permitted to ensure animal welfare and health, under the condition that mechanical aerators are preferably powered by renewable energy sources."*
Article 25h (4) *"The use of oxygen is only permitted for uses linked to animal health requirements and critical periods of production or transport, in the following cases: (a) exceptional cases of temperature rise or drop in atmospheric pressure or accidental pollution,*

(b) occasional stock management procedures such as sampling and sorting, (c) in order to assure the survival of the farm stock."
Article 25s (3c) *"… during fallowing the cage or other structure used for aquaculture animal production is emptied, disinfected and left empty before being used again."*

5.2. Current scientific knowledge

Land based systems

Traditional farms use flow-through systems in which the water is taken in via a damming of the adjacent water course/river. The water then passes through the farm by gravity (i.e., without use of or only minor use of pump energy). Originally the ponds were dug directly into the soil of river valleys close to the stream banks, but some traditional farms have replaced earthen ponds with tanks built of concrete or another waterproof material. Furthermore, various water treatment units are used for mechanical (micro-sieves, settling ponds) and biological filtration of the production water.

Strict environmental regulations have been implemented in the Danish aquaculture sector, which includes feed utilization (FCR) that must be below a certain value. The Danish interpretation about the reference values of the water physiochemical parameters that safeguard the animals' health and welfare, in the organic regulation, is as follows (specified for trout, since water quality is highly species specific): oxygen min. 60%, to be measured daily at all the units and the inlet (not outlet); pH 6-8, to be measured once/week at all the units and daily in the inlet; nitrate max. 300 mg l^{-1}, nitrite max. 5 mg l^{-1}, ammonium max. 5 mg l^{-1}, ammonia max. 0.1 mg l^{-1}, all nitrogenous products must be measured weekly at all the units and daily in the inlet. According to the Danish experience, three different types of model farms were defined based on theoretical calculations of the efficiency of implementing different cleaning technologies in existing traditional farms. However, for various reasons (water abstraction, investment costs, etc.), only two types of the model trout farm were developed (Jokumsen and Svendsen, 2010).

Model trout farms of type 1 are extensive farms with mechanical water treatment and reuse of water (maximum 50 to 100 m^3 water per kg fish produced). A quite efficient internal conversion of nutrients occurs, and the stocking density is relatively low. Water treatment takes place partly by internal conversion processes and partly via sludge cones, micro-sieves (or contact filters), plant lagoons, and sludge basins. Biofilters are not required (Jokumsen and Svendsen, 2010).

Model trout farms of type 2 are intensive farms with mechanical and biological water treatment, with lower water consumption and increased re-use of water compared to model farms type 1. In addition to the internal conversion of nutrients, water treatment occurs via sludge cones, micro-sieves (voluntary), biofilters, and sludge basins, but no plant lagoons are required. However, no Danish trout farm has been converted to this type, perhaps due to the high costs of conversion compared to the obtained increase in feed allowance.

The model farms of type 3 represent the highest level of innovation with the lowest consumption of new water. The maximum value is 3.6 m^3 per kg produced fish, but the current intake of fresh water in these model farms is significantly lower and the degree of recirculation has increased accordingly. Thus, the water intake is significantly lower than the water consumption in traditional flow-through fish farms. Furthermore, type 3 model farms have the highest recirculation level (95%) and the most advanced application of recirculation technologies in the treatment of production water. New water (water exchange) for type 3 model farms, is supplied from upper ground water reservoirs (i.e. borehole, springs or drains under or near to the production plant). This means that these farms, in principle, are completely independent of a water supply from a water course, and no weirs and dams are needed in the water course. Thus, they have no impact on the passage of wild fauna by the fish farm.

Cage systems

By far the largest proportion of European aquaculture biomass production takes place in cage cultures as this is the prevailing production system in the salmonid production. The production of fish in cages is increasing globally, with the technology well developed in Europe, parts of South America and China. Cage farming of a range of fish species is fast advancing in South East Asia. Benefits of cage culture are relatively low investment costs, low energy costs, utilise environmental resources and efficient area use, low carbon footprint compared to other production systems (Rosten et al., 2011).

Sea based cage farming encountered some problems during the first years of the relatively short European history. The Norwegian situation was that the farmers were looking for safe and easily accessible locations for their farms, resulting in sites often with very limited recipient capacity. Shallow localities with little weather exposure and low water currents led to organic overload of the sediments under the farms, often leading to anoxic conditions, production of hydrogensulphide (H_2S) and substantial negative effects on the biodiversity. As this was recognized, new regulations to avoid this have been implemented (NS 9410). According to the Norwegian regulations regarding environmental impact and interactions in relation to the sea bottom, each location has to undertake a recipient inspection (known as MOM B) to ensure that the farm is managed so that the environment close to the site is not negatively affected on long term. The standard applies to all cage based farms in Norway, regardless of organic status. The allowed biomass on a location is based on the recipient capacity to handle organic load. During the application stage this has to be modelled through topographic and hydrographic surveys. The farmers have to document the status of the sea bottom annually by undertaking third party NS 9410 inspections. If the organic load from the fish farm aggregate on the sea bottom, mitigation will be required; e.g. reduced biomass on the farm, or total fallowing of the location for a time restricted period. In between generations, a period of fallowing is obligatory to let the locality rest for a minimum of three months (Norwegian Directorate for Fisheries).

Existing Norwegian aquaculture regulations such as NS 9415 can form a robust and sound operational basis for organic cage aquaculture facilities. NS 9415 governs “requirements for the physical design of the installation and the associated documentation. This includes calculation and design rules, as well as installation, operating and maintenance requirements. This standard contains requirements for the physical design of the installation and the associated documentation. There are, for example, requirements for the physical design of all the main components in an installation, functionality after assembly, and how the installation shall be operated to prevent escape”.

Biofouling in cage aquaculture can affect rearing equipment and infrastructure such as the cage nets, support structures and mooring, floats, barges and buoys (Dürr and Watson, 2010). It can affect water quality within the rearing system by limiting water flow via occlusion of the net (Ahlgren 1998; Beveridge 1996; Fitridge et al., 2012), can act as a vector for transfer of disease (Fitridge et al., 2012) and can also lead to cage loading and structural problems (Swift et al., 2006). This structural loading can lead to component damage, functionality and material fatigue and may impact upon the security and overall integrity of the farming unit. Antifouling with chemicals is prohibited, primarily due to the reported toxicity of coatings that contain e.g. copper or zinc (Burridge et al., 2010). To contrast the effects of biofouling, without using toxic chemicals, cage aquaculture has a number of option including i) air exposure of the netting to reduce levels of biofouling, ii) use mechanical or physical net cleaning procedures such as large scale net washers (*ex situ*) or disk or jet based net cleaners (*in situ*), or iii) the use of biological control such as cleaner fish or cucumbers (Dürr and Watson, 2010). However, net changing is more frequently used to limit the effects of biofouling. In situ net cleaning using mechanical water jets, disc cleaners or divers is now an effective and widespread method for dealing with biofouling in cage aquaculture (Fitridge et al., 2012) but its effectiveness is fouling species specific and does carry some risks in terms of potentially increasing the distribution of colonial fouling organisms via fragmentation (Hopkins et al., 2011). Removal of the nets for *ex situ* cleaning on shore is also a robust method for addressing biofouling problems, but frequent net changing procedures can potentially increase the risk of fish escapes and frequent intensive mechanical washes can reduce the lifespan of the net (Fitridge et al., 2012). Heat treatment of the net and also immersion in acetic acid can also be effective for reducing biofouling levels (Guenther et al., 2011). With regard to biological control of fouling organisms, there are several geographically specific species that may be suitable for limiting the extent of biofouling in cage culture. Species such as the red sea cucumber *Parastichopus califomicus* have been shown to be an effective anti-fouling species when reared in duo-culture with Atlantic salmon in net pens (Ahlgren 1998). Work from the mid 90’s has also reported that wrasse can reduce the prevalence of biofouling on nets in salmon farming (Kvenseth 1996), which could be a secondary benefit of using cleaner fish for parasite and lice control in salmon farming.

Pond systems

Carp fishponds represent unique, man-made aquatic ecosystems that are important and integral parts of the landscape (Kořínek et al., 1987; Adámek et al., 2012). Fish ponds are bound with the rural economy in some regions as well as with the landscape because of their large number and often large size. In some areas, centuries old fish ponds are even the major feature of the landscape. Ponds were built in low-lying wetlands or areas with soil conditions too poor to support productive agriculture, sometimes from water logged fields which had earlier been converted from wetlands.

Currently, the main function of most fishponds is the production of fish based on utilisation of the natural production potential of the pond ecosystem. Since the early twentieth century, however, carp (*Cyprinus carpio*) production in ponds has been systematically increased through a variety of management improvements. Current carp pond management practice (which includes fertilisation and supplementary feeding), together with the influence of agriculture and human settlements, has led to a state in which the majority of ponds in Central Europe is considered as eutrophic to hypertrophic aquatic ecosystems (Pechar 2000; Potužák et al., 2007; Všetičková et al., 2012).

Fishpond farmers must cope with many problems related not only to maintaining the health and nutritional status of fish but also to pond water quality (Dulic et al., 2010; Máchová et al., 2010a; Filbrun and Culver, 2013) and pond ecosystem diversity in general. As such, water quality is likely to be one of the main limiting factors for Central European fishpond farming in future (Kolasa-Jamińska 2002; Wezel et al., 2013). Pond water quality is subject to increasingly stringent requirements, requiring pond managers to have a good understanding of the interactions between biological and chemical parameters within the aquatic environment, including fundamental factors at the trophic and saprobic levels such as nutrient and organic content (Máchová et al., 2010b). According to the level of allochthonous loading (i.e. de facto pollution), the shift in water quality can be positive in the case of strong organic loading of the inflow (Svoboda and Koubek, 1990; Masseret et al., 1998; Všetičková and Adámek, 2013) or negative in the case of good water quality inflow (Všetičková et al., 2012). A large amount of detailed information has been published on the impact of nutrients and other allochthonous substances on the pond environment. On the other hand, data on the release of nutrients by fish, particularly as affected by ingested food quality and feeding technique, are limited. Nutrients released in this manner have important implications for the pond's hydrochemical regime, development of natural food resources and for discharged pond water quality, all of them being extremely important for correct functioning of the pond ecosystem and for the efficiency and sustainability of fish farming.

Generally, carp culture comprises three sequential pond stages. Carp are raised sequentially in three groups of ponds which are drained annually: nursing ponds are up to 1 ha in area and 0.5 m deep (and are often used later in the season as wintering ponds with deeper water) and produce the first year's fish; ongrowing ponds up to 10 ha in area are used to raise 2 year old fish; and marketing ponds at least 50-100 ha in area are stocked with 2 year old fish which are raised until they are 3 - 4 years old and have attained a marketable size of 1.5-3 kg.

The typical polyculture is dominated by common carp (*Cyprinus carpio*) stocked at 50-90% of the total, followed by Chinese carps (bighead carp, *Hypophthalmichthys nobilis;* grass carp, *Ctenopharyngodon idella*; and silver carp, *Hypophthalichthys molitrix*) at 10-30% with a few percent of predators (pike, *Esox lucius*; pikeperch, *Stizostedion lucioperca*; European catfish, *Siluris glanis*) and other species such as tench (*Tinca tinca*). Carp represents a dominant species of pond aquaculture in conditions of a temperate zone and it is, on the one hand, an omnivorous species, but, on the other hand, it is distinguished by selectiveness aimed at larger food organisms of zooplankton and zoobenthos, which are the easiest available in a food offer. A large part of mainly primary production thus remains utterly unused. In order that also other components of a food chain in the pond ecosystem are effectively used, a pond aquaculture is based on the principle of polycultural stocks, i.e., even those species which do not, if possible, directly compete with carp in food at all (or in a very limited extent) are stocked (see above).

Fish are harvested by sein nets in summer and early autumn but at final harvest at the end of the year the water level is lowered and sein nets are used to force fish to swim into harvesting basins located inside or outside the pond. Pond yields are relatively low, ranging from 200 to 1,500 kg ha^{-1}, depending mainly on the altitude abobe sea level and climatic conditions. The current low yield of carp ponds is partly also due to their multiple use, and especially from strong pressure from nature conservancy and environmental groups. Sometimes, pond aquaculture may take second or even third place behind biological treatment of water, water retention and nature conservation.

In addition to the better utilization of land resources, fish ponds may contribute to the better management of water resources. They are suitable not only for the production of fish, but also to accumulate water which can be used for irrigation during dry periods. Moreover, ponds support the life of their surrounding biotopes.

In fish ponds, there is an intensive (0.5 – 1.0 cm per year) development of mud rich in nutrients and organic materials. From time to time, the mud should be dried and removed from the pond. If this material is distributed over less fertile land, the fertility of the soil will increase considerably. Consequently, this way of fish farming can support horticulture or the production of other terrestrial plants.

An increasingly popular way of pond utilization is the integration of fish culture with a recreational activity such as angling. Its advantage is that anglers pay for the fish and the fee for catching fish. They also pay for their accommodation, eat at the local restaurants and buy locally made products and souvenirs.

Carp pond polyculture can be integrated with intensive rearing of other fish in facilities connected with a pond. In this case, the flow-through tanks of the intensive system are constructed usually nearby the fish pond. The effluent of the intensive unit is discharged into the pond where the drifted-in fish faeces and other products of metabolism increase the natural fish food production in the same way as manure does. The unconsumed feed particles drifted from the intensive tanks into the pond are directly utilized as feed. If the ratio of the intensive unit and the pond area is determined properly, a high level of water purification can be achieved.

Integration of intensive and extensive fish culture practices in a traditional pond fish farm is also possible. The small wintering ponds can be used as the tanks of intensive production unit, whereas the large fish ponds can support the intensive unit as a mechanical and biological filter. Another way for integration of intensive fish culture with carp pond polyculture is the cage culture. If cages are placed in the fish pond during the production season, wastes from the cages will be utilized by the pond ecosystem, including by the fish that are produced there.

Primary production is the building force of organic matter from inorganic materials by autotrophic organisms when CO_2 is the single or main source of carbon. These organisms are the autotrophic bacteria and plants which production takes place in the course of photosynthesis. This is when plants (phytoplankton, algae and aquatic weeds) use mineral nutrients, CO_2 dissolved in the water and solar energy from which they build up their bodies.

Consumption in a pond is performed by both autotrophic and heterotrophic (animals, fungi and bacteria) organisms. In the dark period plants consume and break down organic compounds which were produced by them in the light period to release energy for maintenance and growth. Consumers cannot produce organic compounds from inorganic, but feed on living or dead organic materials which they break down to release energy or to use as building stones for growth, maintenance and reproduction. According to the size of consumer organisms, macroconsumers and microconsumers are distinguished in the pond ecosystem.

Macroconsumers are mostly animals, both invertebrates (worms, insects, etc.) and vertebrates (fish, amphibians, reptiles, birds and mammals). On the basis of their typical food, they can be herbivorous, carnivorous, detritivorous or omnivorous. Microconsumers, mainly bacteria and fungi, break down complex organic compounds and release inorganic or relatively simple organic materials (Adámek et al., 2014). Hence, microconsumers are decomposers and their performed process is the decomposition.

The success of pond fish culture depends on how the biological cycle is influenced and controlled. The pond food chains start with the primary production and finish with those organisms or organic materials on which they feed. The food chains of fish which feed on phytoplankton or zooplankton are much shorter than the food chains of predator fish.

Phytoplankton represents the ultimate food resources for filtering zooplankton (majority of cladocerans and rotifers; some copepods), benthic filtrators (sponges, bryozoans, bivalves, larvae of some chironomids) and some fish (silver carp and bighead carp). Filtrators also use, to a smaller extent, bacterioplankton and suspended organic particles (detritus).

Qualitative as well as quantitative composition of pond zooplankton is essentially influenced by a grazing pressure of a fish stock but, in the resulting effect, not only by its biomass, but also the density and species composition are applied. Cladocerans of the *Daphnia* genus in particular are exceptionally efficient in the reduction of food resources (phytoplankton, bacterioplankton) for other zooplankton species (Brooks and Dodson, 1965). Biomass of small zooplankton species often significantly decreases in the presence of numerous populations of daphnias which represent their decisive competitors (Kerfoot et al., 1985). Mass occurrence of large planktonic filtrators accompanied by high water transparency thus indicates a weak grazing pressure of a stock due to a low biomass, or mortality, diseases, etc. In case of an unfavourable ratio between nitrogen and phosphorus concentration (nitrogen limitation), favourable conditions for the development of cyanobacterial water bloom are created in a growing season.

A stock biomass in a carp pond influences to a substantial degree qualitative as well as quantitative relations in the zooplankton community. According to its composition it is possible to infer back not only the density of the carp stock but also many other facts that influence and are influenced by grazing pressure of fish stock. An increased biomass of zooplankton can thus indicate unfavourable health status of fish or unfavourable conditions of the environment (oxygen deficit, presence of cyanotoxins, etc.).

Zoobenthos of ponds also represents an important component of food of the fish stock (mainly of two-year-old and older carp). It consists basically (from 90 – 95%) only of oligochete worms and larvae of chironomids. Predation pressure of two-year-old and older carp, which largely represent benthophagous feeding pattern, reduces biomass of macrozoobenthos down to approximately one half and chironomid larvae are more susceptible to carp predation than tubificids (Tubificidae). Quantitative relations in phytoplankton are transferred also to development of zoobenthos in the form of a competition with zooplankton. If zooplankton which reduces phytoplankton is strongly developed, the food offer in the form of so-called “planktonic rain” of plant microorganisms for zoobenthos decreases and vice versa – if zooplankton is grazed up by fish, this food offer increases especially for chironomid larvae. If populations of filtering cladocerans are restored, supply of phytoplankton to the bottom decreases and chironomid larvae are usually replaced by oligochets. Similarly, the release of a food niche caused by an emergence of chironomid larvae in summer may often lead to their substitution by oligochets.

Management measures

Fertilization

Fertilization of ponds is an intervention the objective of which is to supply macro- and micro-nutrients necessary for development of primary production. At present it is considered to represent a means for adjustment of the proportion of nutrients in ponds in order to support natural production. However, a considerable amount of nutrients flowing into ponds is caused by washes from agricultural land. Organic fertilization is applied due to an increased demand of carbon dioxide for maintaining a buffering capacity of water and supporting development of decomposers – bacteria and saprophytic organisms. Cattle manure is mainly used to fertilize ponds but pig and poultry manure is also used in areas where these livestock are raised. However, ponds in many areas are eutrophic from agricultural, industrial or urban effluents or run-off so fertilization may not be required.

Liming

The basis of liming with the use of limestone or burnt lime is mainly to adjust the alkalinity of pond water with the aim of maintaining its buffering capacity in order to stabilize pH values. It is mainly provided by the reaction of calcium with free carbon dioxide when carbonate complex of hardly soluble $CaCO_3$ and relatively easily soluble $Ca(HCO_3)_2$ emerges. Liming neutralizes water with low pH values to a slightly alkalic reaction which is suitable for fish farming. Pond liming considerably contributes to the function of nitrifying bacteria and mineralization of organic matter in the bottom. In order to reduce organic substances from water column, burnt lime is applied on the surface of the water by the end of a growing season with the aim of improving the oxygen regime. Liming is also used as a disinfectant (burnt lime) or a therapeutic (chlorinated lime) measure. Calcium also represents an indispensable biogenic element which is extracted from the pond ecosystem through outflow, infiltration to the bottom and fish harvest.

Supplementary feeding

About 70 - 75% of the nutrition for the fish is from protein-rich natural food (plankton and benthos) with 25 - 30% from supplementary feeding with energy-rich grain (barley, maize, wheat). Supplementary feeding means providing feed with the aim of supplementing nourishment to farmed fish – however, fish nourishment remains based on natural food resources. Supplementary feeding is initiated due to an excessive exploitation of natural food. Increased supplementary feeding causes an increased deposition of fat in fish organism which is of a less favourable dietetic composition with an increased share of saturated fatty acids. From the hydrobiological point of view, supplementary feeding also represents a certain nutrient loading in the pond ecosystem. It applies mainly to phosphorus which is contained in feeds (including cereals) in an excessive amount taking the needs of a fish organism into account (Adámek et al., 1997; Hlaváč et al., 2014).

Wintering and summering

The primary objective of measures, during which the pond bottom is left to dry over a winter or summer period, is to support the mineralisation processes in an organic component of pond mud. Nitrification and transformation of organic phosphorus to phosphate are the main processes that occur during mineralisation. The indispensable importance of wintering and summering also lies in disease prevention. During summering, much more germs are killed than during wintering due to the disinfecting effect of ultraviolet radiation. On the other hand, during wintering, more nutrients are released as the structure of bottom sediments (mud) is more disturbed by freeze and the mineralization thus takes place on larger surface.

Other interventions – mud removal, littoral clearing and plant cutting

Mud removal and littoral clearing is connected with elimination of extremely thick layers of mud resulting from pond siltation mainly due to soil washes from cultivated agricultural land. In cases of mud removal, the restoration of permanent benthic fauna takes up to several years. Temporal fauna (aquatic insect larvae) is able to restore considerably faster, but its diversity is mostly negatively influenced by a poor substrate that does not contain any macrovegetation and that has a low content of organic matter. Consequences of littoral mud clearing are not so drastic for macrozoobenthos since such intervention applies only to marginal zones usually with more extensive hard emerged vegetation. In case of littoral clearing, restoration of the ecosystem is faster since it is supported by nutrient washes and inocula (seeds, eggs, embryos, etc.).

Plant cutting is a measure which is applied on the ponds which are overgrown by dense macrophyte beds. Besides, a part of removed vegetation usually remains in a pond and provides phytophilous fauna not only with a suitable substrate, but it also creates a rich food base during decomposition and often also favourable hydrochemical conditions (Adámek and Sukop, 1995). If interventions are more extensive, it is necessary to remove a larger part of the mown macrophyte biomass since there would be a threat of oxygen deficits during its decomposition.

5.3. Conclusion and research gaps

From 2004 until 2009 Denmark was the only country in EU to have a national, governmental controlled regulation on organic aquaculture (Larsen, 2014). The Danish national rules were more specific than the new common EU regulation 710/2009 (EU, 2009). Hence, the implementation of the EU regulation 710/2009 (EU, 2009) since 1st July 2010 was a challinging task for the Danish authorities (Larsen, 2014). The challenge was to translate occuring less specific rules of the new EU regulation for implementation into practice. In case of possibility of ”open” interpretation of the EU rules the Danish authorities decided to continue following the previous national rules, practice and experience.

A main aim for the revision is to strengthen and harmonize the rules of production and to raise confidence of the consumers to organic production. However, EU covers an extensive geographic area, which migth impose climatic related challenges for organic production systems in rural areas to fulfil the organic principles.

Another important challenge is, that the current regulation is not sufficiently specific and hence allows different interpretations in different countries, i.e. different conditions of control and anti-competitiveness between the countries. The opposite was the aim.

Based on interpretation of the current regulation EC Reg. 710/2009, (EU, 2009) the Danish authorities on inspection of organic aquaculture facilities have prepared a check-list for approving and control of organic production facilities (Larsen, 2014).
The list deals with culture conditions for different species (a.o. salmonids, cod, bass, bream, trubot, grey mullet, eel, sturgeon, carp, perch, pike, tench, catfish, tilapia) regarding land based culture.

For carp, the way the carp pond aquaculture is managed is already quasi-organic and the shift to certified organic farming is not as demanding as it is for some other species. Many common circumstances, which belong among the requirements for organic carp pond farming, fully cope with conventional farming, such as stock density and fertilization limits. Conversion to carp pond organic culture is a process of developing farming practices that encourage and maintain a viable and sustainable aquatic ecosystem. Management techniques, especially when applied to influence production levels and growth rates must maintain and protect fish good health and welfare. Location of land based organic production units must maintain the health of aquatic environment and surrounding terrestrial ecosystems.
The future research activities should be focused on the environmental aspects of organic pond farming to bring and support the arguments about the eco-friendly way of pond production supporting biodiversity of pond ecosystems. Also the issues of regular and steady organic feed (cereals) supply are of extremely high relevance. The necessity of avoidance of hormonal preparations for induced carp and pond fish spawning is still questionable because pituitary glands, which are used for these purposes, may also be of organic origin, if necessary. However current legislation about organic farming principles does not allow this exception.

6. Recirculation Aquaculture Systems (RAS)

6.1. Regulation

Article 25g (1) ”*Closed recirculation aquaculture animal production facilities are prohibited, with the exception of hatcheries and nurseries or for the production of species used for organic feed organisms.*”

6.2. Current scientific knowledge

According to article 2j of the Reg. 889/2008 a closed recirculation aquaculture facility is defined as “*a facility where aquaculture takes place within an enclosed environment on land or on a vessel involving the recirculation of water, and depending on permanent external energy input to stabilize the environment for the aquaculture animals*”. In a closed recirculation aquaculture system (RAS) new water is mainly supplied for filling up and to replace water lost by evaporation. The degree of recirculation can be of about 95% (Jokumsen and Svendsen, 2010).
Intensive RAS are used in aquaculture production to minimize water consumption, as well as the environmental impact of the water discharge. RAS can use the same water many times and hence includes a wide range of waste water treatment devices (Martins et al., 2010;

Dalsgaard et al., 2012). As a matter of fact, the use of RAS disconnects the production from the external environment.
A sketch of a RAS is given below:

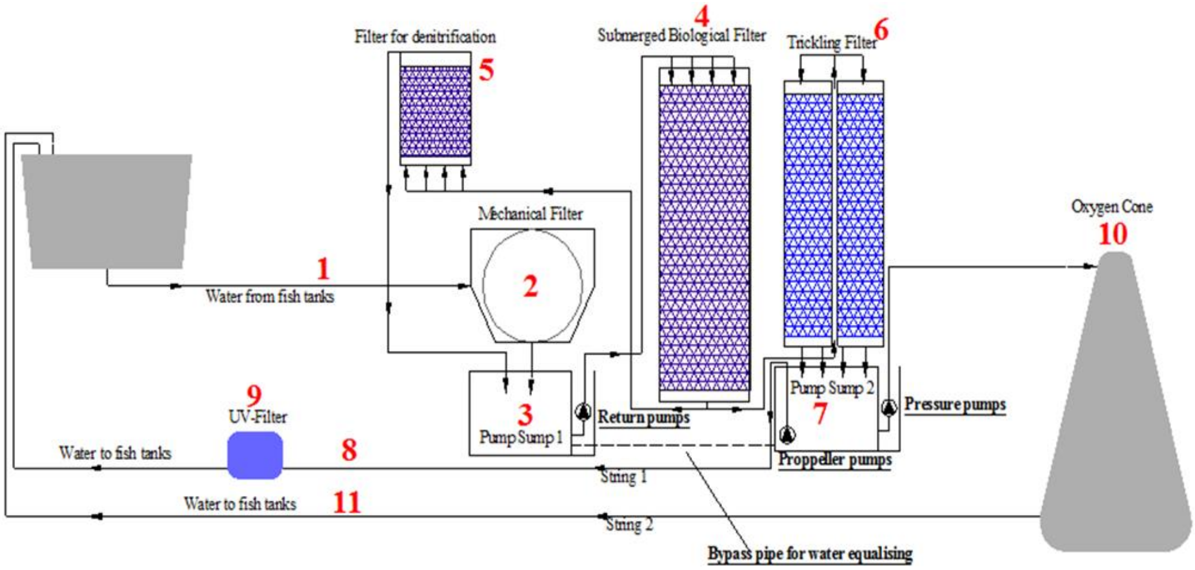

Figure 1: Sketch of a Recirculation Aquaculture System (RAS). The numbers in the figure are referred to (in brackets) in the text. Source: Billund Aquakulturservice ApS, Denmark.

The water supply for an intensive RAS freshwater farm is typically ground water, in the case of marine farms the water is pumped directly from the sea by means of submersible pumps. The production water from the fish tanks (1) passes through a mechanical filter (2), i.e. a micro-sieve (mesh size of about 60 µm). The micro-sieve separates particulate matter, which is flushed as sludge to a sludge storage tank, until it can be used as agricultural fertilizer or for production of biogas. From the micro-sieve (2) the water is pumped (3) to the biofilters (4), where the dissolved fractions, especially ammonia (NH_4^+), are converted into nitrate (NO_3^-). In a separate biofilter (5) with anoxic (no oxygen) conditions (a denitrification filter), the NO_3^- is anaerobically converted into N_2 gas under consumption of easily degradable organic matter (Van Rijn et al., 2006; Suhr et al., 2013). The recirculation water passes on a trickling filter (6) for degassing (N_2, CO_2) and aeration before it enters (7-8) the fish tanks. Before entering the fish tanks, the water passes an UV radiation device (9) to kill micro-organisms, especially bacteria. However, a portion of the aerated water from the trickling filter is pumped through an oxygen cone (7-10) for oxygenation before it enters (11) the fish tanks. In addition, pure oxygen may be added at each tank/section (Chen et al., 2006, Pedersen et al., 2012; Van Rijn, 2013) and the temperature can be adjusted using devices for heating or cooling the water. The amount of new water needed in the RAS corresponds to the amount required to flush the micro-sieves (2) and the biofilters (4), to compensate for evaporation, and to keep the temperature at an appropriate level. The water consumption in RAS is more than 100 times less, i.e. less than 500 l kg^{-1} feed fed to the fish than in traditional flow through systems (Jokumsen and Svendsen, 2010). Obviously, RAS requires input of external energy for pumping water around, water treatment, and aeration of the water, as well as that required in the buildings. The advanced technologies, management, comprehensive surveillance systems, working processes, and hygienic procedures in a RAS farm requires well-educated and trained personnel with the competence required to achieve optimum productivity. The high degree of recirculation makes it critical to continuously monitor and control the water quality within narrow limits, and the extensive use of alarm systems is necessary for several parameters (Jokumsen and Svendsen, 2010).

In the following table, a comparison has been set up between a traditional flow through system in organic farming and an intensive recirculation aquaculture system (RAS).

FLOW-THROUGH ORGANIC SYSTEM	RAS
Advantages • Production in common with nature • Favours biological diversity and animal welfare • Natural temperature and light conditions • Lower stocking density • Behavioural needs can be met • Renewable energy use, e.g. for aerators • Environmentally sustainable **Disadvantages** • Dependent on external conditions (weather, temperature fluctuations, water quality) • Risk of escape • Risk of ingress of pathogens	**Advantages** • Low water consumption • Recycling of water • Stable farming conditions/water quality • Control of water temperature • No environmental impact • Prevents ingress of pathogens • Prevents escapes • Recycling/collection of waste nutrients (fertilizer) • Easy to disinfect/clean **Disadvantages** • Energy consuming • Use of pure oxygen • Higher stocking density • In case of disease, risk of boosting prevalence

Re-use of water

An alternative strategy is re-use of water which, to some extent, combines the advantages of both flow through systems and RAS, without compromising organic principles. Re-use of water means a kind of extensive recirculation in out-door systems with up to 70% of reuse of the water (Colt, 2006). Instead of being discharged, the water is pumped back to the inlet and re-used in the fishponds, tanks or raceways after passing waste water treatment devices such as natural-filter beds, settlement ponds, mechanical or biological filters to collect waste nutrients, and/or using seaweeds and/or bivalves and algae, which contribute to improving the quality of the effluent. The type(s) and capacity of waste water treatment device(s) depend(s) on the specific conditions of the farm, which in turn are related to the production capacity/intensity approved and the fulfilment of water quality criteria.

To comply with the species-specific physiological requirements of the fish, the proper oxygen saturation in the aquatic environment shall be achieved only by using mechanical aerators. This means that there should be a well-balanced equilibrium between the stocking density, the efficiency of the waste water nutrients removal and the amount of water re-used for the proper operation of the organic farm.

6.3. Conclusion and research gaps

Most of traditional organic farms are open-air flow through systems. However, due to the limitations of water resources, national regulations in some countries require that farms are only allowed to take a limited amount of new water from the water courses. In such cases the re-use of water could be a solution in line with the principles of organic production.

Closed recirculation systems (RAS) have several environmental advantages, but require significant input of external energy, high stocking densities (for economic reasons), advanced waste water treatment devices, use of UV radiation and use of pure oxygen. All the above, together with the disconnection of the aquaculture production from the external natural aquatic environment, makes the closed recirculation systems (RAS) not in line with the principles of organic production.

7. Mussel and oyster culture

7.1 Regulation

Article 25n (1) *“Bivalve mollusc farming may be carried out in the same area of water as organic finfish and seaweed farming in a polyculture system to be documented in the sustainable management plan. Bivalve molluscs may also be grown together with gastropod molluscs, such as periwinkles, in polyculture.”*

Article 25n (2) *“Organic bivalve mollusc production shall take place within areas delimited by posts, floats or other clear markers and shall, as appropriate, be restrained by net bags, cages or other man made means.”*

Article 25n (3) *“Organic shellfish farms shall minimise risks to species of conservation interest. If predator nets are used their design shall not permit diving birds to be harmed.”*

Article 25o (1) *“Provided that there is no significant damage to the environment and if permitted by local legislation, wild seed from outside the boundaries of the production unit can be used in the case of bivalve shellfish provided it comes from: (a) settlement beds which are unlikely to survive winter weather or are surplus to requirements, or (b) natural settlement of shellfish seed on collectors. Records shall be kept of how, where and when wild seed was collected to allow traceability back to the collection area. However, seed from non-organic bivalve shellfish hatcheries may be introduced to the organic production units with the following maximum percentages: 80 % by 31 December 2011, 50 % by 31 December 2013 and 0 % by 31 December 2015.”*

Article 25o (2) *“For the cupped oyster, Crassostrea gigas, preference shall be given to stock which is selectively bred to reduce spawning in the wild.”*

Article 25p (1) *“Production shall use a stocking density not in excess of that used for non-organic shellfish in the locality. Sorting, thinning and stocking density adjustments shall be made according to the biomass and to ensure animal welfare and high product quality.*

Article 25p (2) *“Biofouling organisms shall be removed by physical means or by hand and where appropriate returned to the sea away from shellfish farms. Shellfish may be treated once during the production cycle with a lime solution to control competing fouling organisms.”*

Article 25q (1) *“Cultivation on mussel ropes and other methods listed in Annex XIIIa, Section 8 may be eligible for organic production.”*

Article 25q (2) *"Bottom cultivation of molluscs is only permitted where no significant environmental impact is caused at the collection and growing sites. The evidence of minimal environmental impact shall be supported by a survey and report on the exploited area to be provided by the operator to the control body or control authority. The report shall be added as a separate chapter to the sustainable management plan."*

Article 25r *"Cultivation in bags on trestles is permitted. These or other structures in which the oysters are contained shall be set out so as to avoid the formation of a total barrier along the shoreline. Stock shall be positioned carefully on the beds in relation to tidal flow to optimise production. Production shall meet the criteria listed in the Annex XIIIa, Section 8."*

Article 25s (1) *"The animal health management plan in conformity with Article 9 of Directive 2006/88/EC shall detail biosecurity and disease prevention practices including a written agreement for health counselling, proportionate to the production unit, with qualified aquaculture animal health services who shall visit the farm at a frequency of not less than once per year and not less than once every two years in the case of bivalve shellfish."*

Article 25s (2) *"Holding systems, equipment and utensils shall be properly cleaned and disinfected. Only products listed in Annex VII, Sections 2.1 to 2.2 may be used."*

Article 25s (3) *"With regard to fallowing: (a) The competent authority shall determine whether fallowing is necessary and the appropriate duration which shall be applied and documented after each production cycle in open water containment systems at sea. Fallowing is also recommended for other production methods using tanks, fishponds, and cages; (b) it shall not be mandatory for bivalve mollusc cultivation; (c) during fallowing the cage or other structure used for aquaculture animal production is emptied, disinfected and left empty before being used again."*

Article 25s (4) *"Where appropriate, uneaten fish-feed, faeces and dead animals shall be removed promptly to avoid any risk of significant environmental damage as regards water status quality, minimize disease risks, and to avoid attracting insects or rodents."*

Article 25s (5) *"Ultraviolet light and ozone may be used only in hatcheries and nurseries."*

Article 25s (6) *"For biological control of ectoparasites preference shall be given to the use of cleaner fish."*

Article 38a (*1) "The following conversion periods for aquaculture production units shall apply for the following types of aquaculture facilities including the existing aquaculture animals: (d) for open water facilities including those farming bivalve molluscs, a three month conversion period.*

Article 38a (*2) "The competent authority may decide to recognize retroactively as being part of the conversion period any previously documented period in which the facilities were not treated or exposed to products not authorized for organic production."*

Article 79a "When the control system applying specifically to aquaculture animal production is first implemented, the full description of the unit referred to in Article 63(1)(a) shall include: (a) a full description of the installations on land and at sea; (b) the environmental assessment as outlined in Article 6b(3) where applicable; (c) the sustainable management plan as outlined in Article 6b(4) where applicable (d) in the case of molluscs a summary of the special chapter of the sustainable management plan as required by Article 25q(2)."

Article 79c *"For bivalve mollusc production inspection visits shall take place before and during maximum biomass production."*

7.2. Current scientific knowledge

Shellfish culture

In Europe, main shellfih species produced are the Pacific oyster *Crassostrea gigas,* the blue mussel *Mytilus edulis* and the Mediterranean mussel *Mytilus galloprovincialis.* Shellfish farming requires proper shelter, water quality and feed availability in the water. In addition, the nearby presence of spats and suitable substrate are important. These conditions are usually found in coastal waters. In France, the main producer of the Pacific oyster, cultivation of bivalves is practised from Normandy coast to Mediterranean lagoons, facing with various environmental conditions affecting their physiological activity and growth rate. In fact, Pacific oyster is tolerant for salinity and temperature with good growth with ranges between 25 - 32 ppt (tolerance from 10 to more than 35) and 15 - 25°C (tolerance from –2° to 35°C), respectively. Various cultural practices are used in the production of the Pacific oyster, according to the production areas; Foreshore growing with bottom culture (sown oysters) and off-bottom culture (tables), deep waters growing with sown oysters or suspended ropes (typical of mediterranean area), and suspended cultures with floating systems. Pacific oyster juveniles are obtained either through wild spat collection or by hatchery production. Concerning the European flat oyster (*Ostrea edulis*), total european production was 2,200 T in 2012 (FAO, 2015), with main production in France and Spain (around 1000 each) and small scale productions in Ireland and UK.

In Spain, bivalve production has focussed on Mediterranean mussel with around 200,000 T produced in 2010; France is the european second producer, with 75,000 T of both Mediterranean mussel and blue mussel, then Italy (60,000 T) and Netherlands (50,000 T). Mussel production can be on-bottom or off-bottom (raft, bouchots, longlines) but traditionally is based on offshore longlines or intertidal bouchots (mussel beds). Mussel seeds are only provided by natural larvae catchments. After metamorphosis, the larvae turn into spats which require a surface to fix on. During favourable periods (March-April), spats are gathered in the environment and set generally on coconut ropes located offshore or along the intertidal zone. After 2 to 4 months, the catch ropes are moved to rearing structures, offshore longlines or coastal bouchots.

Cultured shellfish and their associated rearing structures (ropes, tables, longlines) may impact the environment in positive and negative ways (Cranford et al., 2006); Four basic areas of concern are the effects of bivalve culture on: (1) suspended particles, particularly in terms of food resources; (2) sediment geochemistry/benthic habitat; (3) nutrient cycling; and (4) benthic and pelagic population dynamics/community structure. Appropriate stocking density for extractive aquaculture should be linked to the carrying capacity of the production area. Carrying capacity is defined by the environmental conditions of the site (trophic capacity, hydrodynamics), the cultivated species (filtration rates, sizes) and the cultural practices (rearing volume, estimated total biomass, stocking densities) of the area. Some models are developed to evaluate the carrying capacity of production areas; those models can predict responses in terms of bivalve growth rate in relation to the different management strategies, taking into account biomasses, species and environmental conditions (Heral 1993). For example, ECASA project (www.ecasatoolbox.org.uk) identified models that can be used to minimize environmental impact from bivalve aquaculture operations.

Benthic community

There is hardly any scientific research into organic culture of mussels and oysters. The main research topics on conventional production focus on carrying capacity of the environment Newell, (2004) and sites Laing and Spencer, (2006), husbandry and farm management, and polyculture with marine fish. One of the environmental risks of shellfish culture is the disruption of the benthic community at the site and this is one of the main topics of shellfish research. Borja et al. (2009) showed that the use of benthic indicators such as individual abundance, and the biomass, together with dynamics of the site, water depth, years of farm activity, and total annual production, are important aspects when interpreting the response of benthic communities to organic enrichment from aquaculture. In a similar study, Christensen et al. (2003) found reduced sediments and enhanced benthic mineralization, containing low microphytobenthic biomass and few subsurface macroinvertebrate species below a mussel farm. This was associated with sulfidic sediments and a lower nitrogen removal rate due to impeded benthic photosynthesis and denitrification activity. Hartstein and Rowden, (2004) showed differences in macroinvertebrate assemblage composition inside and outside of mussel farm sites experiencing different hydrodynamic regimes. The study indicates there is a relationship between the hydrodynamic regime of a farm site, organic enrichment of seabed sediments by mussel biodeposits, and a subsequent modification of the macroinverebrate assemblages. Miron et al. (2005) studied sediments and macroinvertebrate diversity underneath suspended mussel lines in a shallow water system to underline various relationships between benthic parameters and husbandry practices. No strong relationship between husbandry practices and the studied benthic parameters were found and this might be related to the oceanographic characteristics and land-based activities associated with the water system rather than direct and cumulative effects of mussel culture. Mirto et al. (2000) studied the impact of organic loads under a mussel farm and found accumulation of chloroplastic pigments, proteins and lipids, and changes in meiofaunal density. Such changes in the sedimentary conditions reflected the accumulation of faeces and reduced conditions. However, comparative analysis of the mussel bio-deposition and fish farm revealed that mussel farms induced a considerably lower disturbance on benthic community structure compared to fish farms.

In contrast, Danovaro et al. (2004) investigated the impact of a large mussel farm on the benthic environment for one year using biochemical, microbial and meiofauna benthic indicators and concluded that mussel farming in the investigated system is eco-sustainable and does not significantly alter the coastal marine ecosystem.

Byron, (2011) states that ecosystem-based management (EBM) is an important tool to protect coastal ecosystems where bivalve culture potentially pollutes the benthic community. EBM can be improved when it is informed by ecological science and considers the socio-economic needs of the community, and in addition, communication between stakeholders is key.

Carrying capacity and site selection

Jiang and Gibbs, (2005) investigated the carrying capacity of suspended bivalve culture. Introducing the large-scale bivalve culture resulted in a decrease in the mean trophic level of the ecosystem, an increase in the total yield and the bivalves replaced zooplankton as the major grazers in the modelled system. McKindsey et al. (2006) outlines four hierarchical categories of carrying capacity for bivalve farms: physical, production, ecological, and social carrying capacity. At present, most scientific studies focussed on production carrying capacity, however, further knowledge should include better modelling of feedback mechanisms between bivalve culture and the environment, a consideration of all steps in the culture process (seed collection, ongrowing, harvesting, and processing), and culture technique.

In a study to assess the scope for growth of bivalve culture, Gibbs, (2007) presented indicators for interaction between the culture and the water-column. Sea-based suspended bivalve culture development is more often controlled by the ability of the water-column environment to supply particulate food material then by the ability of the sea bottom to absorb waste products. Saxby, (2002) reviewed a range of influences (temperature, salinity, particulate matter, food availability, current speed and water depth) for bivalve growth and condition in a series of areas to build a broad picture of the environmental characteristics of successful bivalve farms. Sedentary bivalve molluscs can tolerate a wide range of water conditions, although fluctuations in environmental conditions influence growth and flesh conditions strongly.

Camacho et al. (1995) showed that site selection, and thus phytoplankton availability, and the choice of seed stock is of major importance in the duration of the cultivation process for mussel culture. In a similar study, Cubillo et al. (2012) studied the effects of stocking density on growth performance on mussels and found that mussels cultured at lower densities showed better growth performance. The effects started to be visible after four months into the culture cycle and implies competition for food and space allocation. The combination of stocking density and seed seize was studied by Lauzon-Guay et al. (2005). A study by Ramón et al. (2007) showed that the using mussel seed from the same area where the cultures are carried out is advantageous for growth and reduced mortality. Rosland et al. (2011) present a model for simulation of flow reduction, seston depletion and individual mussel growth inside a longline farm, to cope with the challenge to configure the farms to optimise production and individual mussel quality under different environmental regimes. The model can be incorporated as a decision support tool in mussel farm management These studies show that farm management can make a significant difference in successful bivalve culture. A statement that is confirmed by Ferreira et al. (2007), who described a model for farm management and regulation.

Larval culture

Larval culture is an important aspect in bivalve culture, where natural seed collection is still relied on in the culture process. Walter and Liebezeit, (2003) investigated spat collectors to improve the process of collecting spat from the environment. Hatchery production could potentially contribute to a reliable and economic expansion of the industry and to genetic domestication. Galley et al. (2010) studied elements of hatchery production of mussel larvae as an alternative for natural spat collection; density, temperature and microalgal diets. Laxmilatha et al. (2011) conducted a similar study where the Asian green mussel was spawned and the larvae were successfully reared, whereas Lazo and Pita, (2012) performed a study with temperature treatments on mussel larvae. Although further studies are needed, these studies highlight that hatchery seed production can produce spat for the industry so that the pressure on the environment decreases.

Polyculture

Some studies also focus on the integration of mussel and oyster culture and cage farming of marine salmon, sea bass and sea bream (Cheshuk et al., 2003; Lefebvre et al., 2000; Navarrete-Mier et al., 2010; Gao et al., 2006). The shellfish can potentially limit the pollution from the fish farms and serve as additional production animals for consumption. In addition, Whitmarsh et al. (2006) examined the financial viability of a polyculture system that integrates the farming of salmon and mussels. The results demonstrated the commercial potential under present market conditions, but highlight the critical role played by future price trends.

7.3. Conclusion and research gaps

According to Mansfield, (2004), shellfish culture at sea is in a way comparable to vegetable crops, in that they are planted and do not migrate. However, farmed shellfish are in other ways similar to wild fish, since they live in open water and feed on organisms in the water, providing no state of control to the producers over the feed (other than maybe the site selection), and thus organic feed is not possible to provide.

According to the actual organic legislation, seed from non-organic bivalve shellfish hatcheries may be introduced to the organic production units with 0% by 31 December 2015. This could be extremely restrictive, both for oysters because organic hatcheries are still not really developped, and for mussel as well, because mussel seeds are collected from natural areas.

As defined by the ICES and FAO codes of conduct for responsible fisheries, oyster and mussel sustainable production is linked to the carrying capacity of the environment; shellfish excretion can impact local sediment and associated populations (both animal and macrophyts). Carrying capacity of the production areas has to be evaluated to defined appropriate stocking density for shellfish aquaculture.

As organic farms shall minimise risks to species of conservation interest, transfer of shellfish has to be better controled, to avoid the risk of alien, translocated species, or diseases introduction. Risk assessment methodologies could be applied to minimize the impact of transfers and to prevent the introduction of invasive species.

8. Seaweed culture

8.1 Regulation

Article 6b (1) *"Operations shall be situated in locations that are not subject to contamination by products or substances not authorised for organic production, or pollutants that would compromise the organic nature of the products."*

Article 6b (2) *"Organic and non-organic production units shall be separated adequately. Such separation measures shall be based on the natural situation, separate water distribution systems, distances, the tidal flow, the upstream and the downstream location of the organic production unit. Member State authorities may designate locations or areas which they consider to be unsuitable for organic aquaculture or seaweed harvesting and may also set up minimum separation distances between organic and non- organic production units."*

Article 6b (3) *"An environmental assessment proportionate to the production unit shall be required for all new operations applying for organic production and producing more than 20* *tonnes of aquaculture products per year to ascertain the conditions of the production unit and its immediate environment and likely effects of its operation. The operator shall provide the environmental assessment to the control body or control authority. The content of the environmental assessment shall be based on Annex IV to Council Directive 85/337/EEC (*). If the unit has already been subject to an equivalent assessment, then its use shall be permitted for this purpose."*

Article 6b (4) *"The operator shall provide a sustainable management plan proportionate to the production unit for aquaculture and seaweed harvesting."*

Article 6b (5) *"Aquaculture and seaweed business operators shall by preference use renewable energy sources and re-cycle materials and shall draw up as part of the sustainable management plan a waste reduction schedule to be put in place at the commencement of operations. Where possible, the use of residual heat shall be limited to energy from renewable sources."*

Article 6b (6) *"For seaweed harvesting a once-off biomass estimate shall be undertaken at the outset."*

Article 6d (1) *"Seaweed culture at sea shall only utilise nutrients naturally occurring in the environment, or from organic aquaculture animal production, preferably located nearby as part of a polyculture system."*

Article 6d (2) *"In facilities on land where external nutrient sources are used the nutrient levels in the effluent water shall be verifiably the same, or lower, than the inflowing water. Only nutrients of plant or mineral origin and as listed in Annex I may be used."*

Article 6d (3) *"Culture density or operational intensity shall be recorded and shall maintain the integrity of the aquatic environment by ensuring that the maximum quantity of seaweed which can be supported without negative effects on the environment is not exceeded."*

Article 6d (4) *"Ropes and other equipment used for growing seaweed shall be re-used or recycled where possible."*

Article 6e (1) *"Bio-fouling organisms shall be removed only by physical means or by hand and where appropriate returned to the sea at a distance from the farm."*

Article 6e (2) *"Cleaning of equipment and facilities shall be carried out by physical or mechanical measures. Where this is not satisfactory only substances as listed in Annex VII, Section 2 may be used."*

Article 29a (1) *"If the final product is fresh seaweed, flushing of freshly harvested seaweed shall use seawater. If the final product is dehydrated seaweed, potable water may also be used for flushing. Salt may be used for removal of moisture."*

Article 29a (2) *"The use of direct flames which come in direct contact with the seaweed shall be prohibited for drying. If ropes or other equipment are used in the drying process they shall be free of anti-fouling treatments and cleaning or disinfection substances except where a product is listed in Annex VII for this use."*

Article 36a (1) *"The conversion period for a seaweed harvesting site shall be six months."*

Article 36a (2) *"The conversion period for a seaweed cultivation unit shall be the longer of six months or one full production cycle."*

Article 73a *"When the control system applying specifically to seaweed is first implemented, the full description of the site referred to in Article 63(1)(a) shall include: (a) a full description of the installations on land and at sea."*

Article 73b (1) "Seaweed production records shall be compiled in the form of a register by the operator and kept available for the control authorities or control bodies at all times at the premises of the holding. It shall provide at least the following information: (a) list of species, date and quantity harvested; (b) date of application, type and amount of fertiliser used."

8.2. Current scientific knowledge

Seaweeds are marine macroalgae, of ecological importance for oxygen production and as primary function in the food chain. Seaweeds are robust and can take up high levels of nutrients and in some cases heavy metals from the water (Chan et al., 2006). The largest and fastest growing species is *Macrocystis pyrifera* (giant kelp). Not surprisingly therefore, this species has been the subject of research for many years. The most common production is by long line cultivation, where sporelings are produced in a cooled water greenhouse and later planted out in the ocean attached to long lines (ropes). Harvesting occurs after two years, by transporting the ropes to the shore for harvesting the entire organism. An alternative harvesting method is by surface canopy harvesting several times each year. Farming of seaweeds is thus either (semi) controlled at sea, or in land based systems. Seaweeds feed on nutrients dissolved in seawater and can be farmed without supplemental feed, a practice called extractive aquaculture.

Seaweeds are cultured for different purposes, mainly as bio-filter for the effluent water in (intensive) aquaculture systems, as feed for other aquaculture products (e.g. sea urchins, shrimp, abalone, fish), as bio-fuel or as human food product or cosmetics.

Some cultivated seaweeds have very high productivity, can absorb large quantities of nutrients (such as N, P, and CO_2) and can produce large amount of oxygen. Culture of seaweeds can thus limit eutrophication that forms a big problem in many coastal regions in the world. There is little information on the influence of seaweed culture on the environment and possible pollution of seaweed farming (Beveridge et al., 1997). Studies mainly focus on the inhibition of eutrophication and the associated algae blooms in coastal areas (Liu et al., 2009; Lüning and Pang; 2003; Fei, 2004) with a focus on the coast of Chile and South-East Asia. Seaweed farming can be particularly problematic for the environment when a proper planning and management in terms of plant density, harvesting strategies and disease control is lacking (Gutierrez et al., 2006). In addition, Ólafsson et al. (1995) and Eklöf et al. (2006) studied the effects of seaweed farming on a sea grass ecosystem and found a decreased biomass, increased litter and a different and poorer fauna in plots where seaweed was farmed compared to controls. In addition to the seaweed, predation of fish and disturbance of the sediment were factors that influenced the environment.

One of the most promising uses of seaweeds is in the production of biofuels as partial replacement of fossil fuels. In fact, both macro-algae (Alvarado-Morales et al., 2013; Dave et al., 2013) and micro-algae (Rajvanshi and sharma, 2012; Singh and Gu, 2010; Brennan and Owende, 2010) are considered to be a promising alternative for fossil fuels due to their availability, growth rate, and oil yields (Langlois et al., 2012). However, it is clear that in spite of the progress and research, some challenges remain (van Hal et al., 2014), before large scale production of biofuel from algae can compete with fossil fuels.

Some research is conducted for seaweeds to be incorporated serve as novel (food) products for humans. This requires specific farming conditions, such as site and sporophyte selection, culture density (Gutierrez et al., 2006), and farm management aspects, such as sustainability and quality assurance. Many seaweeds with the potential for human use are small in seize and occur in low, seasonal densities, forming additional challenges for commercial farming. On the other hand, on-land farming offers the highest level of control (Bolton et al., 2009) (Hafting et al., 2012). Harvested products that are not suitable for human usage can for instance be used for abalone feeding. *Ulva* (Chlorophyta) is cultivated for human consumption in some countries (mainly in South-East Asia) and can grow unattached in estuaries in high nitrogen waters, and secure a large biomasses production and thus more nutrient removal.

Robertson-Andersson et al. (2008) investigated the use of *Ulva lactuca* in a recirculation system for abalone farming and found no adverse effects on the abalone with respect to health or growth rate or the seaweeds by running the system at 25% recirculation, with both cultured organisms behaving similarly to ones cultivated in a flow-through system. In addition, no negative environmental effects from accumulated and suspended sediment build up were reported. In a similar study, (Neori et al., 1998) esthablished an integrated on-based culture of abalone that aimed to eliminate external feed dependency and to reduce water requirements and nutrient discharge levels. Effluents from two abalone (*Haliotis tuberculata*) culture tanks drained into macroalgae (*Ulva lactuca* or *Gracilaria conferta*) culture and biofilter tanks, where nitrogenous waste products contributed to the nutrition of the algae. The net algal production from each algal tank was harvested and used to provide a mixed diet for the abalone. In a study by Msuya and Neori, (2008), intensive fishpond effluent passed through seaweed tanks at four nutrient loading levels and four tank designs for water exchange, bottom aeration and frequently changing water levels to study optimal conditions for *Ulva lactuca* as biofilter. Neori et al. (2003) studied the problem of ammonia kinetics in seaweed based biofilters since seaweed yield and protein content is inversely related to ammonia uptake efficiency, which is in sharp contrast to the desired situation of a high uptake rate in combination with a high uptake efficiency. Sanderson et al. (2008) studied the potential of seaweeds in the vicinity of fish farm cages to maximise potential utilization by cultured macroalgae for nutrient removal from the salmon cages. Additional studies are still needed if the objective is to maximise exposure of cultured algae to farm-derived nutrients, but it shows the potential of the use of seaweeds in aquaculture.

The most studies that involve seaweeds are about seaweed based IMTA systems (Integrated Multi-Trophic Aquaculture, where the seaweed serves as bio-filter and exceed seaweed can serve as feed for the primairy aquaculture product (e.g. abalone or fish).

Abreu et al. (2011) investigated *Gracilaria* as being efficient biofilters and noted that with appropriate upscaling *G. vermiculophylla* can be implemented in fish productions systems with economic and environmental advantages. In a similar study, Hernández et al. (2005) studied the culture of *Ulva rotundata* and *Gracilariopsis longissima* in effluents from an intensive marine grow-out culture of gilthead seabream. Since nutrient limitation in an effluent tank from a fish farm is highly unlikely, self-shading is often the factor that determines maximum stocking density of the seaweeds in biofilter tanks. Kang et al. (2011)

evaluated the potential of *Ulva pertusa, Saccharina japonica* and *Gracilariopsis chorda* as biofilters or effluents from black rockfish (*Sebastes schlegeli*) tanks and showed that the three species can each serve as biofilter. In addition, the study provides information on the behaviour of integrated cultures for upscaling. Mata et al. (2010) investigated the use of the tetrasporophyte of *Asparagopsis armata* has as a novel seaweed biofilter for IMTA and states that the growth and biofiltration rates for this species are much higher than the values described for the most common seaweed biofilter, *Ulva rigida*. More extensive information can be found in the chapter below (10, IMTA).

8.3. Conclusion and research gaps

Most seaweed is produced not for direct human use, there is not much attention for organic production of seaweeds. In general, seaweed production is seen as environmental friendly and sustainable. Therefore there is not much research to organic production of algae.

There is sufficient knowledge present on the use of seaweeds as biofilter, less information is present on the use as feed for aquaculture products, impact on the environment, biofuel and the use for human (food) products. Production of seaweed is considered to have only a low impact on the environment, so not much research is conducted on this.

There is not much scientific information on harvesting issues and on farm management.

However, many articles in the regulation concern farm management issues (administration, production) and not directly linked to the production systems. Production is mainly linked with IMTA, together with abalone, where the seaweed is cultured in the system growing on nutrients from the abalones, and the macro algae on their turn serve as food source for the abalones.

9. IMTA

9.1. Regulation

According to Reg. EC 889/2008,

Reg. 889/08; tittle II CHAPTER 1a, art. 6d 1 *"Seaweed culture at sea shall only utilise nutrients naturally occurring in the environment, or from organic aquaculture animal production, preferably located nearby as part of a polyculture system"*

Reg. 889/08; art. 25n 1 *"Bivalve mollusc farming may be carried out in the same area of water as organic finfish and seaweed farming in a polyculture system to be documented in the sustainable management plan. Bivalve molluscs may also be grown together with gastropod mollusc, such as periwinkles in polyculture"*

Reg. 834/07 (11) (11) *Organic farming should primarily rely on renewable resources within locally organised agricultural systems. In order to minimise the use of non-renewable resources, wastes and by-products of plant and animal origin should be recycled to return nutrients to the land*

Part of this review is linked to review on "Environmental impact and waste recycling"

Reg. 834/07; art. 5 (c) *the recycling of wastes and by-products of plant and animal origin as input in plant and livestock production.*

9.2. Current scientific knowledge

Integrated multi-trophic aquaculture (IMTA) refers to the integration of cultivated species from different trophic positions or nutritional levels in the same system. Chopin and Robinson (2004) distinguish IMTA from polyculture, which could simply be the co-culture of different fish species from the same trophic level. The IMTA approach combines fed aquaculture (fish) with extractive inorganic aquaculture (micro- macro-algae, plant) and/or extractive organic aquaculture (suspension and deposit feeder). IMTA is a practice in which the by-products (wastes) from one species are recycled to become inputs (fertilizers, food and energy) for another.
The genera of interest for IMTA systems in marine temperate waters have been listed by FAO (2009) and include:
– Seaweeds: *Laminaria, Saccharina, Sacchoriza, Undaria, Alaria, Ecklonia, Lessonia, Durvillaea, Macrocystis, Gigartina, Sarcothalia, Chondracanthus, Callophyllis, Gracilaria, Gracilariopsis, Porphyra, Chondrus, Palmaria, Asparagopsis and Ulva.*
– Mollusks*: Haliotis, Crassostrea, Pecten, Argopecten, Placopecten, Mytilus, Choromytilus and Tapes*.
– Echinoderms: *Strongylocentrotus, Paracentrotus, Psammechinus, Loxechinus, Cucumaria, Holothuria, Stichopus, Parastichopus, Apostichopus and Athyonidium*.
– Polychaetes: *Nereis, Arenicola, Glycera and Sabella.*
– Crustaceans: *Penaeus and Homarus.*
– Fish: *Salmo, Oncorhynchus, Scophthalmus, Dicentrarchus, Gadus, Anoplopoma, Hippoglossus, Melanogrammus, Paralichthys, Pseudopleuronectes and Mugil.*

Numerous studies have been carried out in temperate and tropical regions. Nearly hundred experimental studies on integrated tropical mariculture have been published in peer-reviewed journals for the last three decades (FAO, 2009).
The concept of IMTA meet Organic Aquaculture principles on various aspects:

- based on trophic interactions between species, it mimics natural ecosystem
- decrease nutrient load through conversion to valuable biomass
- the co-cultivated species utilise natural nutrients

9.2.1 Nutrient recycling and bioremediation

Seaweed finfish integration:

There are several projects using marine micro and macroalgae as biofilters for waste water from land-based and open cage system (Troell et al., 1997, review FAO, 2009). Several studies on land-based IMTA system have demonstrated their interest in terms of bioremediation and waste valorisation. For instance, Neori et al. (1996) have shown the capacity of *Ulva* sp. ponds to reduce the concentration of dissolved nitrogen from sea bream farm. Pagand et al. (2000) have demonstrated that the high rate algal pond technique may be used for the treatment of marine aquaculture effluents from semi-intensive system with high nitrate concentration. They showed that 60% of dissolved inorganic nitrogen and dissolved reactive phosphorus were removed, although the efficiency varies greatly with season (from 18% in winter to 98% in summer), Biomass production of macroalgae was evaluated to 15 g DW m^{-2} d^{-1} and the potential removal of dissolved N and P (0.15 and 0.015

g m^{-2} d^{-1}). Metaxa et al. (2006) found also that, when *Ulva* and *Cladophora* were used in HRAP, the waste water had significant reductions in the dissolved inorganic N and P. The authors also noted that the algae had no effect on fish growth. Martinez-Aragon et al.,2002 and Hernandez et al. (2002) found that *Ulva rotundata*, *Ulva intestinalis* and *Gracilaria gracilis* co-cultivated with seabass were efficient biofilters for phosphates (PO_4^{3-}) and ammonium (NH_4^+). As demonstrated by Borges et al. (2005), microalgal species (*Isochrysis galbana, Tetraselmis suecica* and *Phaeodactylum tricornutum*)could be efficiently reared in fish (e.g. sea bass and turbot) effluents and reduced the amount of NH_4, NO_3 and PO_4. In Canada, an IMTA system combining salmon, bivalve and kelp have shown an increase of 46% of the growth of the Kelp cultured in proximity to the fish farms, compared to reference sites (Chopin et al., 2004). However, several projects testing fish-algae integration in open system have not observed effect on macroalgae cultured at farm site compared to reference site.

Limit: "The nutrient removal capacity of algae may vary with species, but is rather low compared to the emissions of nutrients from the farms (Abreu et al., 2011). Efficient removal of all nutrient emissions will thus depend on large scale seaweed farms, which would be difficult to place close to fish farms without altering the current pattern and thereby oxygen conditions in the fish farm" (ICES, 2012)

Bivalve and finfish

Bivalves have a high filtration capacity and have been used in IMTA systems to the reduce nutrient load coming from finfish farming. the main species used in IMTA system are mussels and oysters. Increased reported growth rates of bivalves cultured close to a fish farm relative to a reference site may be due to a number of confounding factors (current regimes, shelter, etc.). It is necessary to use an appropriate design or appropriate tracers (such as stable isotope) to better evaluate the efficacy of the mitigation strategy and the proportion of fish effluents used and assimilate by the co-cultured species. Results of studies were reviewed by ICES WGEIM (2012). Jones and Iwama (1991) co-cultured of the oyster *Crassostrea gigas* and the chinook salmon (*Oncorhynchis tsawytscha*) in Canada. Growth rates of oyster were three times higher at the salmon farm site and decreased with distance from the farm.

Mazzola and Sara (2001) estimated that the particulate organic carbon waste from the fish feed provided 80% of the diet of adult clams, *Tapes* sp., cultivated in baskets adjacent to fish pans at 9 m depth, 1 m from the seafloor, and 50% of the mussel *Mytilus galloprovincialis*' diet (suspended on a long-line at 3 m depth). Stirling and Okumus (1995) have also shown that the growth of mussels *Mytilus edulis* was greater at a salmon farm site in Scotland than in a mussel monoculture. They argued that higher POM and *Chl*-a levels, at the salmon farm, may support mussel energy retention during the winter. POM derived from fish aquaculture only contributed significantly to mussel growth during periods of low plankton production, which tend to occur in winter. During this season, shellfish placed close to fish cages could benefit from the additional POM and overcome growth restrictions in winter (Troell et al., 2003). However, other studies have observed no differences in growth rates between fish farm and reference sites, possibly due to: 1) POM loading from fish cages being too diluted (Cheshuk et al., 2003), 2) co-cultured species being grown at too great a distance from farm sites (e.g. 70 m distant in Cheshuk et al. (2003)), and 3) fouling organisms (e.g. mussel, tunicates, polychaetes) growing on the net and farm structures intercepting the POM before it reaches the co-cultivated species. Navarrete-Mier et al. (2010) studied mussel (*Mytilus* *galloprovincialis*) and oyster (*Ostrea edulis*) growth close to finfish farms (sea bream and sea bass) in the Mediterranean and found no differences between farming sites and reference stations 1800 m away.

9.2.1. Interactions between co-cultured species: disease and parasites

It is important to consider the interactions between species in IMTA system. Co-cultured species may either transmit diseases or act as bioremediator. To prevent disease transmission, IMTA species should be are native to the area. Few studies have evaluated the effect of co-cultured species in IMTA system (ICES, 2012).

Tan, (2002) showed that biofouling should be considered as a risk factor for Amoebic gill disease (AGD) outbreaks, as it may be a significant reservoir of the amoebic disease and may contribute to its spread. Skar and Mortensen, (2007) studied the uptake of the pathogenic infectious salmon anaemia virus (ISAV) in mussels, to investigate its potential trans-mission from fish to mussels and vice versa. Viruses are not regarded as a natural food for bivalves but studies have shown that they may be efficiently trapped in mussel mucus strings during feeding. Mussels were not a likely reservoir host or vector for ISAV and they have been shown to destroy the virus. Ingested pathogens do not necessarily remain viable, as Paclibare et al. (1994) showed that the bacterial pathogen *Renibacterium salmoninarum* is removed and killed by the blue mussel, *M. edulis.* Milanese, (2003) tested the capacity of the marine sponge *Chondrilla nucula* to retain the bacteria *Escherichia coli*. They showed that one square meter patch of this sponge can filter up to 14 l/h of sea water retaining up to 7 × 1010 bacterial cells h^{-1}. They suggested that *C. nucula* is a suitable species for marine environmental bioremediation in integrated aquaculture systems. Molloy et al. (2014) studied the fate of ISAV in mussels, *M edulis* and suggested that little to no viable ISAV particles are present in the mussel tissues.

9.3. Conclusion and research gaps

It is important to co-cultivate species that are ecologically compatible, requiring similar environmental conditions and do not compete for food and space in an aquaculture system (Kang et al., 2005). In addition, it is necessary to assess the oxygen demand of each component of the system. Heterotrophs may increase oxygen demand and decrease the oxygen budget of the fish culture. Respiration by autotrophs may also consume oxygen, although oxygen production during the day may compensate for night time consumption (Neori et al., 2004). Bio-deposition rates of each component of the system and the dispersal pattern of particulate and nutrients must be determined to evaluate the efficiency of an integrated system and when evaluating the environmental carrying capacity of a site.

It is unclear how to determine nutrients naturally occurring in the environment and nutrients coming from watershed.

Concerning Reg. 889/08; art. 25n 1, there is a lack of information on co-culture between bivalves and organic fish or seaweed. In addition, there is a lack of information between possible disease/parasite positive and negative interactions between species composing the IMTA system.

10. Ethical aspects related to production systems

One step in the production process where unnecessary suffering could occur is the last phase in the fish life, crowding in tightened nets, pumping to the slaughter house and other handlings before the actual slaughter. Given that unnecessary suffering is not defined in terms of human ends justifies all animal suffering, it is also proven possible to improve. The advice is to avoid waiting cages, but if not possible, good water quality and correct levels of oxygen makes a valuable difference for animal welfare (in terms of health) as well as pumping speed and height (Espmark et al., 2012). Another example of unnecessary suffering is the slaughter by use of CO_2, as this has proven to be inefficient and to cause stress/suffering. It is forbidden in Norway nowadays, but still used in many other countries, and still accepted by the EU.

In addition to the above mentioned aspects of animal welfare, a few comments on differenet stages of the production process are given in the following section. Without doubt all stages of fish production have the potential to cause impaired welfare, and many scientific papers not only describe, but also suggest solutions, or possible steps to improve. Thus, there seems to be a general awareness about welfare impairment among researchers, which raises the question of how this is communicated with the industry and other stakeholders.

This leads to the question of implementation and interpretation of current legislation – is there room for a wide range of interpretations, or is any of these - in a strict sense - illegal? Although there seems to be a need for harmoisation, the EU covers an extensive geographic area, which might impose climatic related challenges for organic production systems in rural areas to fulfil the organic principles. Another important challenge is that the current regulation is not sufficient specific and hence allowing different interpretations in different countries, i.e. different conditions of control and anti-competitiveness between the countries. The opposite was the aim. However, along with such strive for harmonisation, the power of improvement, engagement for better sustainability and animal welfare might be lost. To what extent would a higher level of harmonisation of Organic Aquaculture gain those countries having a ‘stricter than necessary’ interpretation and those countries that operate ‘just on the limit’ respectively? Is there a need for ‘fore runners’ or pioneers in order to inspire the rest and push them, or lies the power in the common effort?

Stocking density (both during transport and production) and behaviour?

In some of the reviewed studies on stocking density, regulation of maximum thresholds are recommended. It is also showed that researchers are aware of some difficulties related to assessment of welfare, not only due to differences between species, large variation in transport and farming conditions, but also in the assessment of welfare. For example on what to measure – mainly external aspects such as water quality and temperature, feeding method, rearing system and environmental conditions, or also internal physiological reactions by the fish (Ashley 2007). In both cases results are to be interpreted and compared with something else in order to evaluate what is ’too high’ or ’too low,’ and it has proven difficult to find a comomon scale or zero point to relate to. From an ethial point of view, one can ask if this tricky situation could call for a ’benefit of the doubt thinking’, taking precautionary measures? It seems though that the discprepancies are so large between how welfare in relation to stocking density is measured, that also this is very difficult. Given that aggression seems to be at lowest in both low and high stocking density, finding ’safe’ levels is hard (Brockmark et al., 2007; Cañon Jones et al., 2011). On the other hand, it may seem hard, because economic aspects are implicitly taken into account in a ’would be too expensive with low density, or much more space’- thinking. But, what if good welfare pays off, as can often do in other animal husbandry, in terms of better health, lower veterinary costs, better growth rate etc? Would an argument along the line of benefit of the doubt be more plausible? In addition, a risk assessment process would need to be performed, stating known risks and assessing the plausibility that they occur, predicting the risk of unknown risks, and discussing different handling strategies.

Further, the three-dimensional welfare definition (biological functining, subjective expereiences and natural behaviour) becomes relevant again. If arguing along the lines of other organic husbandry, that natural behaviour is important for the individual animal’s welfare, a wider range of issues need to be included and related to each fish species concerned - from reproduction behaviour, to feed and the possibility to swim in a ’natural pattern’, for e.g. salmon at distances rather than depths, which leads to questions about size of the basin or cage.

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Chapter 4: ENVIRONMENTAL IMPACT

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1. Introduction and current regulations

1.1. Introduction

The rationale behind organic food production is to minimise the impact of the production on the environment. The global food sector is currently responsible for around 30% of the world's energy consumption and contributes to more than 20% of the global greenhouse gas (GHG) emissions (FAO 2011a). In addition, land use changes contribute (mainly deforestation) to another 15% of GHG emissions. The Food and Agricultural Organization (FAO) projects that 70% more food needs to be produced globally by the year 2050 to feed a world population of 9 billion people and calls for urgent action in developing food systems that use less energy and emits less greenhouse gases (FAO 2011b). At present, there are few standards or threshold values for what can be defined as sustainable food production. However, in recent years there is increasing interest for developing models, metrics and tools to measure environmental impact. Sustainability indicators are being recognised as a useful tool for policy making and public communication in environmental performance (Pelletier et al., 2007; Singh et al., 2009). The main purpose of environmental indicators is to summarise, focus and condense the complexity of our environment to a manageable amount of meaningful information which will provide decision-makers with a tool to determine which actions should be taken to make food production more sustainable.

1.2. Current Regulation

710/2009 Article 6b 5: "*Aquaculture and seaweed business operators shall by preference use renewable energy sources and re-cycle materials and shall draw up as part of the sustainable management plan a waste reduction schedule to be put in place at the commencement of operations. Where possible, the use of residual heat shall be limited to energy from renewable sources*".

710/2009 Article 25 h 3: "*Aeration is permitted to ensure animal welfare and health, under the condition that mechanical aerators are preferably powered by renewable energy sources*". Energy from renewable sources is defined as renewable non-fossil energy sources: wind, solar geothermal, wave, hydropower, landfill gas, sewage treatment plant and biogases regulation

834/2007 Article 15 1b.iii: *"husbandry practices shall minimise negative environmental impact from the holding, including the escape of farmed stock"*
889/2008 Article 25f.4: *"Containment systems shall be designed, located and operated to minimize the risk of escape incidents."*
889/2008 Article 25f.5. *"If fish or crustaceans escape, appropriate action must be taken to reduce the impact on the local ecosystem, including recapture, where appropriate. Documentary evidence shall be maintained."*

EC Regulation 889/2008 25 q 2 Section 6 Specific rules for molluscs. (see chapter 3 Production systems, paragraph 8.2).

834/2007 Article 15 1b.iii: *“husbandry practices shall minimise negative environmental impact from the holding, including the escape of farmed stock”*
EC regulation 889/2008, article 25g 3: *Containment systems at sea shall: (a) be located where water flow, depth and water-body exchange rates are adequate to minimize the impact on the seabed and the surrounding water body.*

834/2007 Article 5(c): *In addition to the overall principles set out in Article 4, organic farming shall be based on the following specific principles: (c) the recycling of wastes and by-products of plant and animal origin as input in plant and livestock production;*

No 710/2009 Article 6b 5: *Aquaculture and seaweed business operators shall by preference use renewable energy sources and re-cycle materials and shall draw up as part of the sustainable management plan a waste reduction schedule to be put in place at the commencement of operations. Where possible, the use of residual heat shall be limited to energy from renewable sources.*

No 710/2009 Article 6d 4: *Ropes and other equipment used for growing seaweed shall be re-used or recycled where possible.* (see chapter 3 Production system, paragraph 9).

2. State of the art on energy use and LCA

2.1. Current regulations

710/2009 Article 6b 5 “*Aquaculture and seaweed business operators shall by preference use renewable energy sources and re-cycle materials and shall draw up as part of the sustainable management plan a waste reduction schedule to be put in place at the commencement of operations. Where possible, the use of residual heat shall be limited to energy from renewable sources*”.

710/2009 Article 25 h 3 “*Aeration is permitted to ensure animal welfare and health, under the condition that mechanical aerators are preferably powered by renewable energy sources”*. Energy from renewable sources is defined as renewable non-fossil energy sources: wind, solar geothermal, wave, hydropower, landfill gas, sewage treatment plant and biogases regulation

There are no specific regulations on the release of CO_2 (carbon footprint) and global warming potential (GWP). The global warming potential is however closely related to the use of fossil energy, so energy use and CO_2 release are treated together in this review.

2.2. Current scientific knowledge

Energy is used in all parts of the production system, from feed raw material production, feed manufacturing, hatchery production, grow-out phase, slaughter and transportation of materials and fish during the entire value chain. Mineral oil is the most important non-renewable energy source used in the production chain. Mineral oil is used in the fishing vessels, farm machinery and for transport processes throughout the value chain. Hydropower are partly used in the production phase (hatcheries, cage farming, and also in feed production. However, in a common European electricity market both power from renewable sources such as hydropower and wind power are mixed with non-renewable energy from coal and natural gas. To be sure of using renewable energy, a hatchery would need to have its own power supply from wind, sun or biogas. Prototypes for biogas production from fish sludge are being tested, but there is still a long way ahead before such operations will be economically viable alternatives to conventional energy sources.

RAS are forbidden in organic production, witht the execption for hatcheries and nurseries, or for the production of species used for organic feed. RAS have a high energy use, but because of re-use of water and on-land facilities, RAS have a low impact on the environment (more on RAS in chapter 3 Production systems).

For salmon, it is important to be aware of that most of the total energy consumed during the production of the final salmon product is used for growing, harvesting, processing and transporting the feed ingredients of the salmon feed (Ellingsen and Aanondsen, 2006, Tyedmers et al., 2007, Ellingsen et al., 2009, Pelletier et al., 2009, Boissy et al., 2011, Skontorp et al., 2011). Thus, the regulations laid down for feed composition in organic salmon production will be a direct driver for how much energy will be used in the production of salmon. The general rules on feed for fish, crustaceans and echinoderms states that feeding regimes should be designed that create low environmental impact (regulation 710/2009 article 25 j) and the rationale behind organic food production is to produce food with the lowest possible environmental impact.

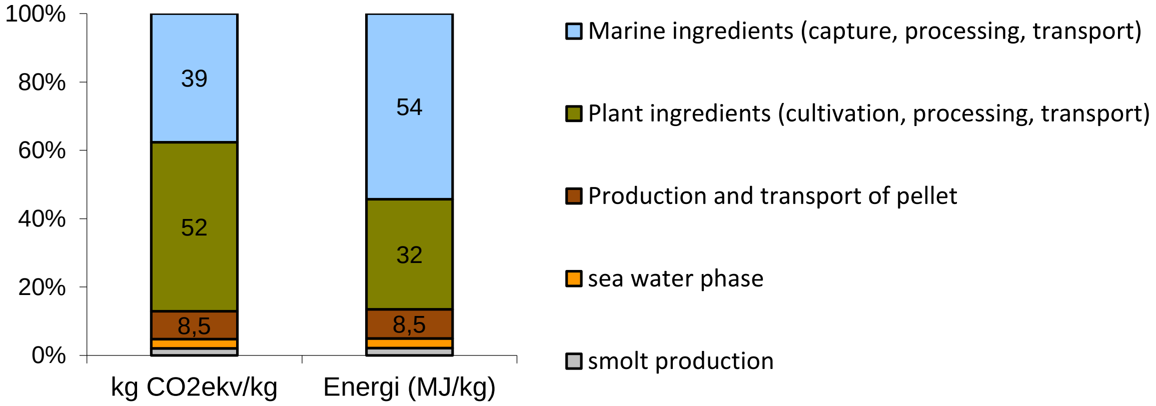

Figure 1: Example of distribution of energy consumption and CO_2 emissions during the salmon production chain, starting with the capture/growing of feed ingredients and ending at the farm gate with 1 kg of edible salmon product. Values are % of total energy use and CO_2 released in the production of 1 kg edible salmon product global at the farm gate (Data from Hognes et al., 2011).

2.2.1. Methods for assessing energy consumption – Life Cycle Assessment (LCA)

Life Cycle Assessment (LCA) is currently the most standardised method for assessing environmental performance. LCA is an ISO-standardized analytical framework for evaluating the environmental impacts of products or processes (eco-efficiency). A life cycle refers to the life span of a product from resource extraction, manufacture, use and final disposal. When complete, a LCA estimates the cumulative environmental impacts resulting from all stages in a product's life cycle. There are two ISO standards specifically designed for LCA application: ISO 14040 (Principles and framework) and ISO 14044 (Requirements and Guidelines). LCA was originally developed to evaluate the life-cycle impacts of industrially manufactured products, but is now increasingly being applied to evaluate food production systems, including aquaculture. The LCA framework is used to quantify the input of energy and resources as well as the environmental impacts associated with each stage of a product's life cycle, from resource extraction and processing, consumption, disposal and recycling. There are two commonly used methodological approaches in LCA: attributional LCA and consequential LCA. The former focuses on describing a product system and its environmental exchanges using with a retrospective point of view. Consequential LCA describe how the environmental impact of a system can be expected to change as a result of actions taken in the system, (for example a change in feed composition or production regimes) and thus reflects the possible future impact of a change in production (Samuel-Fitwi et al., 2013).

The main phases of an LCA are described as follows:

- Definition of the goal and scope of the analysis
- Inventory analysis - making a model of the product life cycle with data collection of all environmental inputs and outputs
- Impact assessment – the effects of the resource use and emissions generated are grouped into impact categories which may be weighted according to their importance
- Interpretation – The results of the inventory analysis and impact assessment are discussed, and conclusions are drawn

The primary goal of a LCA is to select the product alternative with the least harmful effects on human health and the environment. If two products are to be compared, a unit for comparison is also defined (the functional unit). Usually, the functional unit is a defined volume or mass unit, for example a kg of live animal or a kg of edible product. To trace absolutely all inputs and outputs from a system is impossible, so boundaries around the system must be defined. This is a critical step that may have large impact on the outcome of the study. An important question is whether the production and disposal of capital goods (trucks, factory equipment, fishery vessels, net pens for fish farming etc.) are included in the analysis. In modern databases capital goods are usually included, and in general, capital goods should be included if they give a significant contribution to the outcome of the LCA. When agricultural systems are analysed it must be defined whether the agricultural land is seen as a part of nature or as a production system. In the inventory analysis, the products lifecycle is defined and all material and energy requirements as well as all emissions to air, water and soil are quantified. During the impact assessment, the magnitude of environmental impact from different processes is quantified by using impact categories that represent environmental issues of concern. The impact may be on both global and/or local scale. The potential impacts are modelled using conversion factors to obtain one indicator for each impact category. An impact category may for instance be the global warming potential, where all gases that contribute to global warming (CO_2, N_2O, CH_4) are converted into CO_2 equivalents based on their global warming potential. Table 1 shows examples of impact categories and category indicators. An important task when evaluating environmental impacts is to identify which processes that contribute most in the outcome of the LCA. This may for instance be certain life cycle stages (e.g. feed production), certain impact categories (e.g. global warming), or certain inventory parameters (energy use). Most LCA studies performed on aquaculture productions so far have excluded environmental costs associated with infrastructure, seed production, packaging and processing of product, transport of feed and product, cooking of product and disposal of waste. This is due to the fact that the bulk of the environmental emissions and consumption of resources is related to the production phase and to feed production.

Table 1: Impact categories and category indicators often used in LCA analyses

Impact category	Scale	Category indicators
Climate change	global	Global warming potential expressed as CO_2 equivalents
Ozone depletion	global	Ozone depletion potential expressed as CFC-11 equivalents
Acidification	regional/local	Acidification potential expressed as SO_2 equivalents
Eutrophication	local	Eutrophication potential expressed as PO_4 equivalents
Toxicity (human/ecosystem)	global/continental	Contributes to conditions toxic to marine flora/fauna Expressed as 1,4-DCB equivalents
Photochemical oxidant formation	local	Photochemical ozone formation potential, expressed as ethylene (C_2H_4) equivalents
Land use	global/regional/local	Land occupation, expressed as m^2/year
Biotic resource use	global	Appropriation of net primary productivity (NPP) carbon appropriated
Abiotic resource depletion	global	Depletion of minerals and fossil fuels, expressed as Sb (Antimony) equivalents or MJ for energy use
Water use	Global, regional, local	Expressed as litre/year or litre/kg

2.2.2. Allocation of environmental impacts in LCA

Most food production processes has several outcomes. Thus, flows of materials and energy as well all emissions must be allocated between the different products. The ISO standard recommends avoiding allocation by either sub-dividing the process in two or more separate processes to isolate the component of interest, or to expand the system boundaries to include processes or products that would be needed to make a similar output. The environmental burden from the alternative processes is then subtracted from the original system. This approach is called the avoided burden method.

If it is not possible to avoid allocation, the ISO standard recommends allocating the environmental load according to an underlying physical relationship that reflects the material balances between the inputs and outputs of the system. Examples are allocation according to the mass or energy content or economic value of the products and by-products. Allocation based on mass or energy content reflects the biophysical flows through the production system and is stable over time. Economic allocation is not stable over time because the prices of products may change in response to changing availability and market demands. The choice of allocation method is one of the most controversial methodological issues in LCA because it has a large impact on the outcome of the LCA (Ayer et al., 2007, Svanes et al., 2011, Pelletier and Tyedmers, 2011) and the ISO standard states that a sensitivity analysis should be performed if there are several alternative allocation methods that may be applied.

In fisheries and aquaculture productions allocation may become necessary in the fishing stage, during processing of feed ingredients and feed production. The economic value of main product and co-products has been used to allocate impacts between main product and by-product and express the relative importance of an output. However, using economic allocation may change the outcome of the analysis if the price of the products changes. Mass allocation divides the contribution to environmental impact equally according to the mass of the main product and by-product. Trimmings and by-products have a lower economic value than the fillets for human consumption, but may represent more than half of the total weight and contain a major proportion of the total energy content of the fish.

The choice of allocation method is clearly important if by-products form fisheries or livestock productions are used in salmon feed production. In mass allocation, the environmental cost associated with the by-products is the same as for the products for human consumption. Thus, the use of by-products from "environmentally costly productions" such as livestock production in salmon feed production will contribute substantially to the outcome of an LCA analysis in terms of energy use and CO_2 emissions. Using mass allocation in LCA's is beneficial for producers of products for human consumption if they can recycle their by-products into other production systems. This may create an incentive for avoiding dumping or burning of processing waste. On the other hand, economic allocation is more beneficial for the consumer of by-products and creates an incentive for using these valuable resources.

Despite the problems with co-allocation in LCAs, system expansion or splitting up the process in several sub-processes to avoid allocation has rarely been applied in LCA studies involving sea food products (Ayer et al., 2007). Samuel-Fitwi demonstrated how system expansion may be used in assessing environmental effects of farming rainbow trout (Samuel-Fitwi et al., 2013).

The sensitivity of choice of allocation method and the practice of using mass units as functional units in LCA studies might be the most serious limitations of the model when applied to food producing systems. Using mass as functional unit makes LCA unsuitable for measuring the retention efficiency of nutrients in food production systems. Because the main function of food is to provide nutrients, it would be more useful to use the nutritional value of a product as a basis for comparison between products. The nutritional value of a product may be defined as a sum of all the ratios: (nutrient gained/daily requirement) for a kg of a certain product. The nutritional value has been suggested used as a normalisation factor when assessing impacts of a production (Mungkung and Gheewala, 2007). Energy consumption and global warming potential in salmon farming - summary of knowledge from recent LCA studies

Several studies that have focused on different aspects of Atlantic salmon and salmonid production have been published in recent years. Some of the studies have compared different fish diets and production systems (Papatryphon et al., 2004, Pelletier and Tyedmers 2007, Ayer and Tyedmers, 2009 Pelletier et al., 2009, Boissy et al., 2011, Hall et al., 2011) (Table 2). In aquaculture productions using pelleted feeds with high energy content, production and processing of feed ingredients and feed is particularly resource demanding and it may account for up to 90% of the total energy consumption and environmental impacts of salmon production (Ellingsen and Aanondsen, 2006, Tyedmers et al., 2007, Ellingsen et al., 2009, Pelletier et al., 2009, Boissy et al., 2011, Skontorp et al., 2011). Thus, the composition of the diet and the energy use and CO_2 released when producing the feed ingredients is very important for the cumulative energy consumption and global warming potential of Atlantic salmon production. The feed regulations for organic aquaculture state that maximum 60% of the diet for carnivorous finfish can be of plant origin (from organic crops). The fish meal and oil of the diet should come from trimmings, either from organic aquaculture productions or from sustainable fisheries for human consumption. On a general basis, the use of plant derived ingredients increases terrestrial land occupation but reduces the biotic resource use (measured as net primary production) compared to diets with high levels of marine ingredients (Papatryphon et al., 2004, Boissy et al., 2011). Ellingsen and Aanondsen (2006) compared the energy use in salmon production with production of chicken and wild caught cod and found chicken to be the most energy effective whereas wild caught cod was comparable to farmed salmon. When the marine ingredients were replaced with plant ingredients the energy demand of salmon production was reduced to a lower level than for chicken production. Although fisheries are generally more energy intensive than farming operations, conventional crop production is dependent on nitrogen fertilizer which is highly energy demanding to produce. Furthermore, if forest is removed to make room for agriculture production, the amount of CO_2 released as a result of this change in land use may be attributed to the crop ingredients produced on the land. One example is Brazilian soy, which is given a higher CO_2 footprint than soy from Canada and USA. However, Brazilian soy is mainly non GMO crops, whereas GMO soy is dominating in many other soy producing countries. Brazilian soy is therefore used as ingredient in salmon diets, both conventional salmon and organic salmon.

Plant ingredients are very variable with respect to environmental impacts. Production of camelina oil, for instance, uses more water and energy than the production of rapeseed and palm oil (Boissy et al., 2011). Also, in a study comparing rapeseed oil and palm oil, it was concluded that palm oil was preferable to rapeseed oil in terms of land use, ozone depletion, acidification, eutrophication and photochemical smog whereas it was unclear which oil was preferable in terms of global warming (Schmidt, 2010). Only organic crop ingredients and fish meal and oil from trimmings, either from organic productions or from sustainable fisheries, is approved in organic aquaculture feeds. Organic production of the main ingredients used in conventional salmon diets (canola, soy, wheat and corn) was estimated to consume 60% less energy and reduce the CO_2 emissions by 23% compared to conventional production of these crops in Canada (Pelletier et al., 2008). The reduction in energy use was almost exclusively due to the high energy demand in producing conventional nitrogen fertilizers compared to the biological nitrogen fixation used in organic agriculture.

What kind of ingredients that are used in the salmon feed clearly has a large effect on how much energy the salmon production consumes and thus to a large extent defines the global warming potential of the salmon production. However, it is important to be aware of that how the environmental load is allocated between co-products has a large impact on the outcome of an LCA if by-products from fisheries or animal productions are used as ingredients in the salmon feed. The use of mass or energy content as allocation method, results in a higher environmental impact of feeds containing by-products from land animal productions (Pelletier and Tyedmers, 2007, Pelletier et al., 2009) due to the high input of energy and primary production required to produce livestock. Using economic allocation reduced the average life cycle environmental impacts with 60% for organic salmon diets containing fishery trimmings and organic crop ingredient (Pelletier and Tyedmers 2007).

Because the majority of the energy and global warming potential of salmon production is associated with the feed production, efficient use of this feed for salmon production is obviously very important. Feed efficiency (FCR) is thus a key factor in reducing the cumulative load of environmental impacts (Papatryphon et al., 2004, Pelletier et al., 2009). Selective breeding (Thodesen et al., 2001), farm management practices, diet composition and reduction of production losses are all important factors for reducing the FCR and thus the environmental impacts of salmon farming. Fish meal from trimmings and offal contain les protein, more minerals (15% ash) and has a lower digestibility compared to high quality fish meal produced from whole fish. As a result, the FCR may increase with the when the inclusion rate of by-product fish meal in the diet. If the FCR increases by 10% from 1.1 to 1.2, the carbon footprint of salmon will also increase by around 10% because 90% of the CO_2 emissions in salmon farming are related to feed production and transport of feed.

Table 2: Summary of LCA studies involving cumulative energy use and global warming potential in Atlantic salmon

Authors	Aim	Allocation method	Functional unit	Energy use and global warming potential (GWP)
Pelletier and Tyedmers 2007	Comparing organic and conventional salmon production	Gross nutritional energy content	Production of 1 ton of salmon feed	9900-26 900 MJ/tonne 700-1800 kg CO_2 equiv/tonne
Pelletier et al., 2009	Comparing global salmon farming systems	Gross nutritional energy content	1 ton of live salmon	26 200 – 47 900 MJ/tonne 1793-3270 kg CO_2 equiv/tonne
Ayer and Tyedmers 2009	Compare culture systems	Gross nutritional energy content	1 ton of live salmon	26 900 MJ/tonne 2073 kg CO_2 equiv/tonne
Boissy et al., 2011	Effect of diet formulation Plant ingredients Marine ingredients	Economic	1 ton of salmon feed 1 ton of live salmon	23 803 MJ/tonne 1660-1960 kg CO_2 equiv/tonne
Ellingsen and Aanondsen 2006	Comparison of wild caught cod, farmed salmon and chicken, effect of diet and energy source	Economic and mass	0.2 kg salmon fillet	65 MJ/kg salmon (13 MJ/FU, FU = 200 g fillet)
Ellingsen et al., 2009	Evaluating Farmed salmon production	Economic and mass	1 kg salmon fillet	2.3 kg CO_2 eqiuv/kg
Hall et al., 2011	Comparing global salmon farming systems	Mass allocation	1 ton of live salmon	23 300 MJ/tonne 1290 kg CO_2 equiv/tonne
Hognes et al., 2011	Comparing salmon diets and other meat productions	Mass allocation	1 kg of edible product	25 MJ/kg edible product 2.7 kg CO_2 equiv/kg edible product

2.3. Conclusions and knowledge gaps

A major problem at the moment is the lack of defined criteria and reference points for determining what an environmental sustainable food production is. Thus we do not recommend setting any fixed standards for energy use and global warming potential for organic salmon. Developing methodology for measuring environmental load without allocating environmental effects between products and co-products will benefit organic aquaculture productions like salmon where trimmings from fisheries are used as a feed ingredient. It could also be opened for using fish meal and oil from certified forage fisheries in organic salmon production, because some of these fisheries are very energy efficient. The current regulations say that energy used in the production should preferably come from renewable energy sources. Most of the energy used for production of salmon is diesel oil used in growing and harvesting of feed ingredients. At the moment there is no alternative that can fully replace this energy source, but biodiesel may be an alternative in near future. Still, we do not recommend changing the current regulations.

3. State of the art on escapes from cage culture

3.1. Introduction; current regulation

834/2007 Article 15 1b.iii: *"husbandry practices shall minimise negative environmental impact from the holding, including the escape of farmed stock"*
889/2008 Article 25f.4: *"Containment systems shall be designed, located and operated to minimize the risk of escape incidents."*
889/2008 Article 25f.5. *"If fish or crustaceans escape, appropriate action must be taken to reduce the impact on the local ecosystem, including recapture, where appropriate. Documentary evidence shall be maintained."*

3.2. Current sceintific knowledge

Escapes are a problem for almost all European farmed finfish species. This document will collate and review current scientific knowledge and practices in relation to how to reduce the incidence and prevalence of escapes from aquaculture facilities, in addition to outlining current mitigation and recapture methods if fish escapes were to occur. Where available, the operational efficacy of each action will be reviewed in relation to the above regulations, and knowledge gaps and bottlenecks in the literature will be highlighted. Where appropriate, the review will also incorporate data from national governmental monitoring agencies e.g. the Norwegian Directorate of Fisheries that can be used to evaluate the efficiency of practices that are used to reduce escapes. The greatest body of robust data on escape occurrence, frequency and mitigation is from the Norwegian aquaculture industry, which has some of the best aquaculture legislatory and reporting requirements in Europe (Jackson et al., 2013). The regulatory and husbandry strategies developed within the Norwegian aquaculture industry, especially with regard to cage aquaculture, can therefore be used as a template and framework that other European aquaculture production systems and species can build upon.

Operational methods for reducing the risk of escapes from aquaculture facilities in relation to regulation 889/2008 Article 25f.4

When referring to escapes from cage aquaculture, the definition usually refers to escapes involving juvenile and adult individuals. However, for certain European species such as Atlantic cod *Gadus morhua* L. and gilthead sea bream *Sparus aurata* L. which are known to potentially spawn during the cage holding phase of aquaculture production, escapes of viable gametes via spawning (e.g. Jørstad et al. 2008) has also emerged as an escape risk (see also Dimitriou et al., 2007; van der Meeren and Jørstad, 2009; Somarakis et al., 2013). The following review will therefore discriminate between i) escaping individuals and ii) escapes via spawning.

As stated in the introduction, the most robust data on fish escapes comes from the Norwegian aquaculture industry, which has some of the best aquaculture legislatory and reporting requirements in Europe (Jackson et al., 2013). Official escapes data on salmonids (Atlantic salmon and rainbow trout) goes back to 2001 and escapes data for Atlantic cod goes back to 2004 (Jensen et al., 2010). When reviewing the causes of escapes, Jensen et al., (2010) reported that from 2006 – 2009, 68% of Atlantic salmon escapes were caused by structural factors. These escapes could be directly related to mooring and structural failures, or abrasion and tearing of nets during severe weather events, possibly in tandem with human error in terms of farm operation or installation.

Figure 1 describes the evolution of escapes of Atlantic salmon from Norwegian farm facilities from 2001-2011 and Figure 2 shows the escapes as a percentage of the farmed stock. Figure 1 demonstrates a marked decrease in the number of escapes from 2006 onwards, and this decrease came about at 2 years after a national legislatory Technical Standard NS 9415 was enforced that was specifically formulated for reducing escapes from commercial Norwegian cage salmon farming.

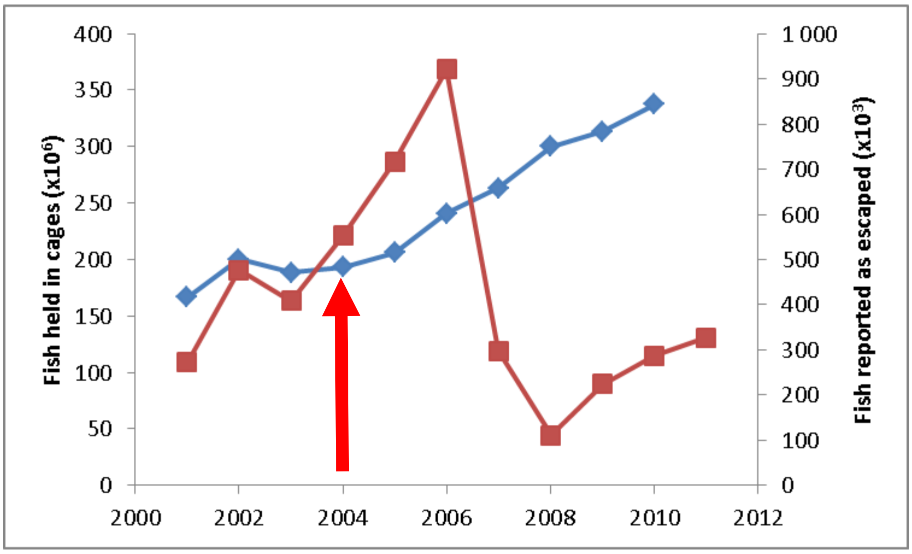

Figure 1. The annual number of Atlantic salmon escapes from Norwegian cage aquaculture facilities from 2001-2011. The red line shows the number of annual escapes (in thousands). The blue line shows the total annual number of fish stocked in cages (in millions). The red arrow shows the year (2004) when Technical Standard NS 9415 was introduced, containing requirements for physical design, installation and management of cages (graph redrawn using data from Jensen et al., 2010 and the Norwegian Fiskeridirekoratet).

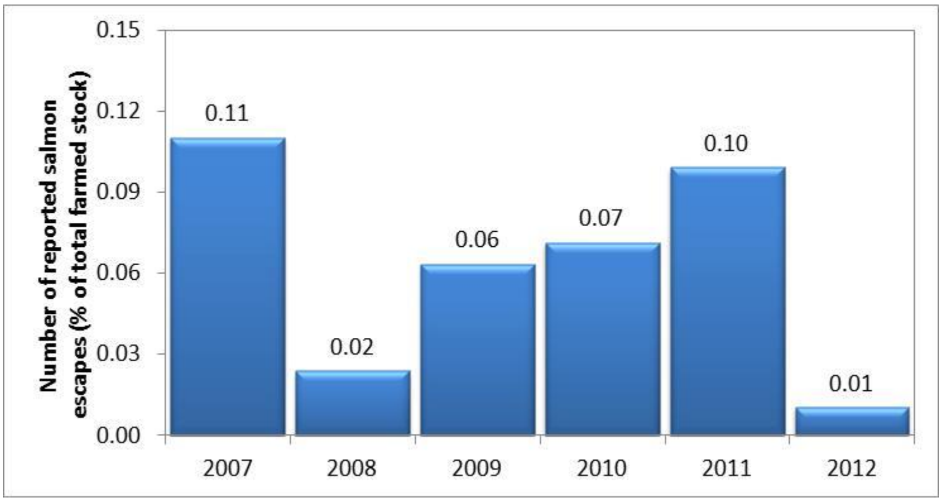

Figure 2. The percentage of escapes in relation to the number of salmon farmed in Norwegian sea cages from 2007 – 2012. Calculated using data from the Norwegian Fiskeridirektoratet.

NS 9415 – potential escape prevention strategy for European aquaculture?

According to the Norwegian Ministry of Fisheries and Coastal Affairs NYTEK Regulations, NS 9415 governs “requirements for the physical design of the installation and the associated documentation. This includes calculation and design rules, as well as installation, operating and maintenance requirements. This standard contains requirements for the physical design of the installation and the associated documentation. There are, for example, requirements for the physical design of all the main components in an installation, functionality after assembly, and how the installation shall be operated to prevent escape.” NS 9415 is also adjusted for given localities. This Technical Standard fits well with the current EU Regulation No. 889/2008 Article 25f.4 states that “Containment systems shall be designed, located and operated to minimize the risk of escape incidents.” NS 9415 could therefore be adopted and refined for organic salmon aquaculture within the EU, and refined for other farming species. However, other cage farmed species such as Atlantic cod and gilthead seabream can demonstrate and exhibit behaviours that increase the risk of escapes, such as biting the net walls and also escaping through holes in sea-cages (Hansen et al., 2009; Glaropoulos et al., 2012). In the first instance, a suggestion would be to use better and more robust netting materials that the standard nylon netting that is currently widely used in aquaculture (after Moe et al., 2007). These can include: Econet/KikkoNet – PET Polyethylene terephthalate; Copper Alloy – copper, zinc alloy (65% copper); Dyneema – High performance polyethylene HPPE; or anti-bite coatings. Other methods that have been shown to be effective in reducing net biting frequency and severity include matching the colour of net repairs to the existing net (Damsgård et al., 2012), and also cage enrichment (Zimmermann et al., 2012), whilst underfeeding increased net biting (Hansen et al., 2009; Glaropoulos et al., 2012). Therefore, simple cost effective management procedures can also reduce the risk of escape in those species that exhibit net biting behavior.

3.3 Conclusions and knowledge gaps

Specific conclusions regarding 889/2008 25f.4

- Species-specific distinctions could be made between escapes of fish and escapes of viable gametes (e.g. Jørstad et al., 2008) within the EU Organic regulations?
- As the majority of juvenile and adult escape events within Europe are attributable to storm damage or the formation of holes in the net walls of cages (see www.preventescape.eu) the EU organic regulations could adopt something similar to Norwegian technical standard NS 9415 which has led to a marked reduction in the severity of escapes from Norwegian cage aquaculture since 2004. NS 9415 governs "requirements for the physical design of the installation and the associated documentation. This includes calculation and design rules, as well as installation, operating and maintenance requirements. This standard contains requirements for the physical design of the installation and the associated documentation. There are, for example, requirements for the physical design of all the main components in an installation, functionality after assembly, and how the installation shall be operated to prevent escape." This standard could include (for each species/production system):
    - Robust regulation and appropriate technical standards for equipment
    - Independent auditing of the implementation of these standards
    - Guidelines for improved staff training, such as the creation of Standard Operating Procedures to limit the frequency and impacts of escapes caused by operational factors.
    - NS 9415 is also adjusted for given localities and therefore fits well with the current EU Regulation No. 889/2008 Article 25f.4.
- Use netting materials that have better resistance to tearing or biting (in the case of Atlantic cod and gilthead seabream). For fish that do exhibit net biting behaviours, do not starve or underfeed the fish for prolonged periods (Hansen et al., 2009; Glaropoulos et al., 2012), match the colour of net repairs to the colour of the existing net (Damsgård et al., 2012) and potentially provide environmental enrichment (Zimmermann et al., 2012).

Operational methods for recapturing escapees from cage aquaculture facilities in relation to regulation 889/2008 Article 25f.5:

It has been suggested that to better protect wild fish stocks from the potential detrimental consequences of aquaculture escapes, attention should be focused on preventing escapes (e.g. Triantafyllidis, 2007; Serra-Llinares et al., 2013). However, if an event were to occur, organic farmers and regulatory bodies must attempt to mediate against the impacts of the escapes by e.g. initiating a recapture and recovery program. A better understanding of the post-escape dispersal of escaped fish can improve recapture efficiency (as it can help focus and direct recapture efforts. For example, fish can disperse rapidly and widely after an escape event and can disperse >1 km from the farm in a few hours (Whoriskey et al., 2006). This rapid migration is not always the case though; it depends on species, life-stage, locality, time of year, and in some cases fish can remain around the farm for weeks (Arechavala-Lopez et al., 2012). However, a recovery program should be initiated as soon as an escape has been discovered to increase the likelihood of potential recapture. The efficacy of recapture methods for i) Atlantic salmon, ii) Atlantic cod and iii) gilthead seabream will therefore be investigated in order to identify potential recommendations and knowledge gaps in relation to regulation 889/2008 Article 25f.5.

Recapture methods for Atlantic salmon

- Chittenden et al., (2011) reported that a coastal bagnet fishery that fishes in the upper level of the water column near the shore can catch up to 69% of released individuals, and rod angling caught another 10%. A total of 39 fish were released in the study.
- Hansen and Youngson (2010) simulated an escape event with the release of 678 fish in Scotland and 597 in Norway. Recapture rates were 0.6% and 7.0% respectively. 64% of the Norwegian recaptures were by rod anglers.
- Skilbrei et al., (2010) reported that from a release of 132 fish, ca. 38% of the escaped salmon were reported as recaptured by recreational fishers. Most were caught by gillnett (77.8%) but others were caught by rod from land (13.9%) and others by bagnets (5.6%).
- Skilbrei and Jørgensen (2010) released 1031 fish. A surface pair-trawl was unsuccessful, and caught only 6 fish. Gill-netting proved to be an efficient method of recapture. 389 fish were recovered from 104 fishers. A total of 94.9% of these were from recreational fishers using gill-nets and this equated to a recapture rate of ca. 35-40%.
- Skilbrei et al., (2014) reported that recapture rates for smolts and post-smolts were low, usually <1% in a large scale release study. Recapture rates of adults were 7-33%. Recapture methods primarily including gillnets and rod fisheries and traditional sea based fishing during summer.
- Tlusty et al., (2008) reported a recall success rate of up to 80% of juvenile Atlantic salmon when they are conditioned to return to a feeding pen via the use of an acoustic signal in a laboratory experiment. This method has not been demonstrated for large fish or at the commercial scale.

Recapture methods for Atlantic cod

- Uglem et al., (2008) reported cod recaptures by local fishermen of ca. 50% during small-scale releases (<80 fish) in a Norwegian fjord system.
- Uglem et al., (2010) reported cod recaptures by local fishermen of 33% during small-scale releases (<45 fish) in a Norwegian fjord system.
- Serra-Llinares et al., (2013) looked at numerous different recapture methods for cod including i) crane-operated dip nets, ii) fyke nets next to cages, iii) static fishing pots and iv) gill nets around the farm locality. The gill nets recaptured <5% of the released fish (ca. 2800 were released) and had high by catch of non-target species. 8 recaptured tagged cod 8 were reported via a reward program for local fishermen during the 6 months post release.

Recapture methods for gilthead seabream

- Arechavala-Lopez et al., (2012) reported that the best way to recapture gilthead seabream after an escape was to use gill nets. Recreational fishing was less successful than commercial fisheries (professional trammel-netters) in recapturing escaped fish.

The authors therefore suggest that local artisanal fisheries should be involved in any recapture program immediately after the escape has been discovered. However recapture efficiency was still low and was less than 10%, irrespective or recapture method.

Specific conclusions regarding 889/2008 Article 25f.5.:

- For species that have the potential to spawn within the cages (Atlantic cod and gilthead seabream), the use of a curtain-like egg collector may be used to mitigate against the occurrence of an egg escape (Somarakis et al., 2013). An aquaculture site planning and locality policy could also be to limit the farming of large seabream that are viable spawners in areas near wild seabream nursery grounds (Somarakis et al., 2013). For Atlantic cod, mandatory harvesting before the second spawning season may markedly reduce the risk of egg escape (Uglem et al., 2013).
- For escapes involving juvenile and adult fish, the majority of all recapture methods are only partially effective, and focus should be on prevention. However, organic farmers can diligently monitor their farms for escapes via a robust and rapid surveillance of the farm infrastructure and fish e.g. during and after extreme weather events or large-scale fish handling, as the first few hours may be crucial.
- Species-specific escape mitigation and recapture plans should be drawn up for organic cage aquaculture.
- Atlantic salmon recapture rates are highly variable but recovery programs that incorporate gill nets (deployed at various depths), rod anglers and also coastal bag net fisheries appear to be the most effective (see Chittenden et al., 2011; Hansen and Youngson, 2010; Skilbrei et al., 2010; Skilbrei and Jørgensen, 2010; Skilbrei et al., 2014).
- Atlantic cod recapture programs are also highly variable but artisanal fisheries and gill nets may be more effective than other methods (see e.g. Uglem et al., 2008, 2010 and Serra-Llinares et al., 2013).
- One of the most robust ways to recapture gilthead seabream after an escape is to use gill nets (Arechavala-Lopez et al., 2012) but they still reported recapture rates of <10%. Organic farmers could have gill nets readily available around a farm in case they need to deploy them and initiate a rapid recapture program.

Knowledge gaps:

- The risks of escapes through spawning from cage aquaculture is currently not well documented for all relevant European species. The potential efficacy of curtain-like egg collectors that can be used to mitigate against the occurrence of an egg escape has also not been tested at a commercial scale (Somarakis et al., 2013)
- Net biting behaviour in Atlantic cod and gilthead sea bream should be investigated further in commercial farm settings, with a particular focus on the efficacy of using environmental enrichment to reduce net biting frequency, which has not been well demonstrated at the commercial scale.
- Current recapture methods may not be a robust tool for recapturing escaped fish, thus hindering the development of a robust species-specific recapture and escapee

recovery program. They may also lead to a high bycatch of none-target species (Serra Llinares et al., 2013). More research is needed on how best to deploy existing recapture methods and practices and develop new ones.

4. State of the art on sea bottom, wild fish feeding and pond water quality

4.1. Current regulation

The current EC regulations in relation to environmental impacts and interactions in relation to sea bottom are as follows:
EC Regulation 889/2008 25 q 2 Section 6 Specific rules for molluscs. (see chapter 3 Production systems, paragraph 8.2)

834/2007 Article 15 1b.iii: *“husbandry practices shall minimise negative environmental impact from the holding, including the escape of farmed stock”*

EC regulation 889/2008, article 25g 3: *Containment systems at sea shall: (a) be located where water flow, depth and water-body exchange rates are adequate to minimize the impact on the seabed and the surrounding water body.*

710/2009 article 25b(4): *“For aquaculture animal production in fishponds, tanks or raceways, farms shall be equipped with either naturalfilter beds, settlement ponds, biological filters or mechanical filters to collect waste nutrients or use seaweeds and/or animals (bivalves and algae) which contribute to improving the quality of the effluent. Effluent monitoring shall be carried out at regular intervals where appropriate.“*

4.2. Current scientific knowledge

4.2.1. Sea bottom detoriation and wild fish feeding

The EC regulations regarding environmental impact and interactions in relation to the sea bottom are not very specific. The organic label Debio which regulates organic production in Norway (and is developed in collaboration with the Swedish KRAV) is based on this EC regulation, but goes further in detailing the requirements. The Debio requirement specifies that no significant organic sediments should build up under the farms. The organic load to the environment (feed waste and faecal material) should be minimised to avoid eutrophication. Each location has to undertake an recipient inspection (known as MOM B) based on the Norwegian Standard 9410 (NS 9410), to ensure that the farm is managed so that the environment close to the site is not negatively affected on long term. The standard applies to all cage based farms in Norway, regardless of organic status.

Debio regulation also states, dependent on technical limitations, that the farm may be required to collect and remove feed waste and faecal material within and around the farm. In fresh- and brackish water, where the background level of nutrients is known, only closed containment systems or adequate systems for faecal waste collectors can be used.

If we look at the general regulations in Norwegian salmon farming, which applies not only to organic production, there are specific requirements that follow the licenses. The allowed biomass on a location is based on the recipient capacity to handle organic load. During the application stage this has to be modelled through topographic and hydrographic surveys.

The farmers have to document the status of the sea bottom annually by undertaking third party NS 9410 inspections. If the organic load from the fish farm aggregate on the sea bottom, mitigation will be required; e.g. reduced biomass on the farm, or total fallowing of the location for a time restricted period. In between generations, a period of fallowing is obligatory to let the locality rest for a minimum of three months (Norwegian Directorate for Fisheries).

Minimising the organic load from the farms means reducing feed waste and faecal material. There is little knowledge regarding the amount of lost feed but it has been assumed to be as high as 5% (Otterå et al., 2009). The loss may be due to uneaten pellets falling through the cages (Dempster et al., 2009) and fragmentation during feeding (Aas et al., 2011). The feeding will follow the growth of biomass, and the waste load will thus be highest during the summer months when production is highest. As the faecal material represents the undigested part of the feed, this proportion depends on digestibility of the feed. The digestibility of aquafeeds for salmon are generally high, with the faecal material representing about 12.5% of the mass of used high energy feed (Kutti, 2008). Introduction of plant materials as replacement of marine raw materials in the feed has reduced the digestibility of the diets. It has, however, also reduced the mechanical stability of the faecal material, leading to higher frequency of smaller particles (Brinker and Friedrich, 2012) which will reduce local sedimentation.

Possible consequences of wild fish feeding and how this relates to organic production practices.

With regard to wild fish feeding there are no EC regulations that applies specifically. In the Debio regulations, however, one of the objectives listed is to protect local wild (fish) populations towards negative effects from the aquaculture activity.

Generally, any floating or underwater device, natural or artificial, may serve as fish attracting device (FAD) (Dempster and Taquet 2004). Attraction of wild fish to open cage farms is a global phenomenon, and more than 160 species belonging to about 60 families have been detected in the near vicinity of such farms (Sanchez et al., 2011 Dempster et al., 2002, 2005; Boyra et al., 2004; Valle et al., 2007; Fernandez-Jover et al., 2008; Halide et al., 2009; Arechavala-Lopez et al., 2010; Fernandez-Jover et al., 2011; Šegvić Bubić et al., 2011; Ozgul and Angel, 2013). Provision of shelter alone may not be regarded negative, even though it can change spatial distribution of the fish.

Marine fish farms may serve as FADs by providing uneaten fish feed, structural habitats and by attracting small prey species (Sanches-Jerez et al., 2011). Waste fish feed, i.e. the feed pellets that is not eaten by the farmed fish and therefore pass through the cages is believed to be the major cause for aggregation of wild fish at sea cage farms (Fernandez Jover at al., 2007; 2010; Dempster et al., 2011, Sanz-Lazaro et al., 2011). As there is some uncertainty as to how much feed that is actually wasted, any estimate of how much feed wild fish eat cannot be accurate; Felsing et al. (2005) reported 40% whereas Vita et al (2004) reported 80%.

The extent of the area around salmon farms within which wild fish are attracted is not known. In the Aegan Sea it has been shown that the spatial structure of wild fish around Sparidae farms was affected at a scale of 10 to 24 squared nautical miles (Giannoulaki et al., 2005). There is some evidence that presence of fish farms in the Aegan Sea not only aggregate fish, but also leads to increased wild fish production possibly due to increased nutrient availability (Machias et al. 2005; 2006).

There are some practical consequences of the aggregation, as the distribution of wild fish in space and time are variable. Whether this is relevant to the organic regulations remains unclear. According to Norwegian legislations, fishing is prohibited closer than 100 metre from the fish farm. As the bulk of wild fish aggregate within 25 metres from the cages (Dempster et al., 2010), this part of the stock is no longer available to commercial fisheries for a large part of the time. On the other hand, as the farms are FAD, abundance of fish can be elevated also outside the fishing-restricted zone providing local fisheries with a predictable source that is less dependent on weather conditions.

The more ecological consequences of FAD and wild fish feeding on waste feed may be regarded as environmental effects, at least in a wider definition. It is well known that farm aggregated species, like Saithe (*Pollachius virens*) will achieve different liver size, lipid content and fatty acid composition in both muscle and liver (Skog et al., 203; Dempster et al., 2009; Otterå et al., 2009; Bustnes et al., 2010; Arechavala-Lopez et al., 2010; Uglem et al., 2009; Fernandez-Jover et al., 2011). In fish, the process of sexual maturation is usually linked to body energy stores, especially fat (Rowe and Thorpe, 1990; Rowe et a., 1991) and large stores at an early age may lead to earlier maturation and a change in recruitment rate (Woodhead, 1960; Bagenal. 1969; Luquet and Watanabe, 1986). High energy levels may also cause increased fecundity and thus higher number of offspring per female (Woodhead 1960).

The amount and composition of fatty acids also affect the quality of the offspring (Watanabe et al., 1984; Izquerido et al., 2001; Bell and Sargent, 2003). Reduced fertilization, as well as quality of egg and larvae are related to nutrition (Sargant et al., 2002). Some essential fatty acids, in particular (arachidonic acid - ARA, eicosapentaenoic acid – EPA, and docosahexaenoic acid –DHA), are important as they may affect fecundity, egg quality and hatching, as well as malformation of marine juveniles (Izquerido et al., 2001; Sargent et al., 1999; Tocher, 2012). Not only the levels but also the ratio can be negative to reproduction and viability of offspring (Izquerido et al., 2001; Morehead et al,. 2001; Cejas et al., 2003; Tveiten et al., 2004; Lanes et al., 2012).

4.2.2. Pond water quality

Supplementary feeding with cereals and its influence on water quality

Common carp are the most frequently cultivated fish in Central and Eastern European ponds (Mráz and Picková 2009; Mazurkiewicz et al., 2011; Mráz et al., 2012). As a traditional culinary fish, its economic importance is enhanced by its ability to adjust to changeable environmental conditions (e.g. water quality), to accept diverse fish feed components, and its high feed conversion ratio (Jauncey 1982). Production in Central Europe is typically achieved using a combination of semi-intensive farming, based on natural food, and supplementary feeding with cereals (Hepher and Pruginin 1982; Moore 1985, Horváth et al., 1992; Kaushik 1993), which represents from around 25–30 % (Adámek et al., 2012) to more than 50% (Tacon and De Silva 1997) of total yield. Supplementary feed has proved a useful tool for providing the nutrients and energy required for improved fish growth and production (Abdelghany and Ahmad 2002), though it has raised concerns as allochthonous substances (feed) are released into the ponds during the production season.

Cereals are one of the most frequently used supplementary feeds in semi-intensive aquacultural ponds (Turk 1995; Zajic et al., 2013), the main components including rye, triticale, maize, wheat and barley (Edwards 2007). While these cannot fully cover the needs of complete carp nutrition, they represent a cheap and readily available source of energy (Turk 1994, 1995; Mráz and Picková 2009). The cereals used tend to have a high proportion of carbohydrates, the primary source of energy for cultured fish (Smith 1989; Sadowski and Trzebiatowski 1995; Sargent et al., 2002). Carp have enzyme systems with high amylase and maltase activity, enabling the fish to utilise large amounts of carbohydrate, though oversupply can result in deposition of fat (Yamamoto et al., 2003; Urbánek et al., 2010). Cereal grains provide the majority of carbohydrate in feeds used in carp nutrition, the proportion amounting to 35–45% of the diet on average (Przybyl and Mazurkiewicz 2004). The larger part, however, consists of starch (60–70%), of which the carp is able to digest around 60–80% in the raw state, depending predominantly on the cereal species (Hernández et al., 1994; Medale et al., 1999; Krogdahl et al., 2005).

However, when grain is subjected to the thermal treatment (roasting, boiling, extrusion), starch becomes gelatinous and its subsequent digestibility may reach up to 90%. Such high carbohydrate digestibility makes grain a basic source of energy in the diet, and this, in turn, allows better utilisation of dietary protein for fish weight gain (Sadowski and Trzebiatowski 1995), in addition to lowering the environmental loading of undigested waste particles. Total protein content in cereal grain varies depending on the species, but ranges between 7 and 15% (Füllner et al., 2000; Kowieska et al., 2011). The protein is poor in essential amino acids needed by fish, however, and thus is of poor biological value (Przybyl and Mazurkiewicz 2004; Másílko and Hartvich 2010). Wheat and other cereals contain antinutritional substances (e.g. albumin) that inhibit aamylase activity (Hofer and Sturmbauer 1985). Others include protease inhibitors, phytoestrogens, goitrogens, antivitamins, phytase and various oligosaccharides and antigenic (allergenic) proteins (Tacon and Jackson 1985; Hendricks and Bailey 1989; Friedman 1996; Alacro´n et al., 1999). These are undesirable as they reduce both feed intake and nutrient bioavailability, which leads to slower growth and higher loading of excreta in the water (Van der Ingh et al., 1996; Alacrón et al., 1999). Due to the thermal instability of some antinutritional factors, however, it is possible to use heat treatment to reduce, limit or inactivate the enzymes responsible without impairing the feed (Másílko and Hartvich 2010). Some antinutritional factors can be present in the hulls of cereals, and therefore, removal of the grain's hull prior to heat treatment can also significantly reduce the impact of these factors with little or no effect on the feed's digestibility (Robaina et al., 1995; Glencross et al., 2007).

Mashing has been shown to improve the feed conversion rate of cereals by between 11 (Másílko et al., 2009) and 18% (Urbánek 2009). Tacon and Jackson (1985) have shown that grinding into meal improves the digestibility of feed by breaking the grain surface, which can also result in a reduction in undesirable antinutritional factors.

The use of good-quality pelleted, and especially extruded, feeds is less expensive and minimises water pollution and the spread of disease through high digestibility and low conversion rate, resulting in better fish growth (Ćirić et al., 2013) and less organic waste loading per kg of fish produced (Cho et al., 2006). The addition of thermal and mechanical treatment prior to their application in carp ponds should further contribute to decreasing the load of undigested or poorly digested supplementary feed.

Concern for the natural environment, and with surface water quality in particular, has led to the imposition of requirements regarding the physical and chemical parameters of both input and output waters of ponds (Fournier et al., 2003). Recently, it has become the norm in most EU countries to allow fish cultivation, provided it does not have an adverse impact on water quality. Some scientists are even of the opinion that, in some cases, the natural significance of fishponds may outweigh their production role (Lymbery 1992; Duras and Potužák 2012). Pond water quality, however, varies throughout the production season, the scale of such changes depending to a large degree on the amount and quality of fertiliser and fish feed used (Diana et al., 1997; Hartman 2012). Intensifying pond fish cultivation by increasing stock density and supplying large amounts of supplementary feed has an obvious impact on environmental conditions in a pond (Kolasa-Jamińska 1994; Szumiec 2002; Abdel-Tawwab et al., 2007). Indeed, according to some researchers (e.g. Seyour and Bergheim 1991; Das et al., 2005; Sindilariu et al., 2009), it is the use of supplementary feed itself that poses the greatest threat to water quality and a number of studies have highlighted adverse impacts on the pond environment from the addition of large quantities of feed, especially where it is improperly balanced or of poor nutritional value (Horner et al., 1987; Poxton and Allouse 1987; Poxton and Lloyd 1989). Strategies aiming to reduce the impact of aquacultural waste, therefore, have to address feed composition, feeding technology and feeding strategy (Cho and Bureau 1997; Bureau and Hua 2010; Hua and Bureau 2012). Feeding strategy improvements are generally based on two main approaches: improving nutrient retention (Cho and Bureau 1997, 2001; Dalsgaard et al., 2012) and increasing waste removal efficiency (Amirkolaie et al., 2005a b, 2006; Lefrancois et al., 2010).

Fishpond water quality is affected through interactions between a range of physicochemical determinants, including temperature, oxygen regime, transparency, nutrient content, pH, alkalinity and hardness, along with the biological component, with fish as a final link in the pond food chain (Ponce et al., 1994; Das et al., 2005; Jana and Sarkar 2005). The physicochemical characteristics of both the pond bottom and the water column are not static, but change with management measures applied, e.g. fish stock composition, supplementary feeding, manuring and fertilisation. Both bottom soil and sediments and pond water quality undergo complex changes due to all these factors (Milstein et al., 2001; Ali et al., 2006; Ahmed et al., 2013). Impact of pond aquaculture on the surrounding environment tend to be more diverse and include physicochemical and biological changes not only due to the fish production system itself but also due to the use of available resources, which can generate conflicts with other end-users (Table 1). Finally, water quality attributes will also be influenced by inputs related to the metabolism of the fish (or other aquatic organism) being cultured (Milstein and Svirsky 1996; Chatterjee et al., 1997; Bechara et al., 2005; Rahman et al., 2010).

During the growing season, the physicochemical and biological parameters of inlet water have been changed by the time it is discharged, due to a number of factors, including stocking density, species composition, management methods, climatic conditions, elevation, original quality and quantity of inflow water, hydraulic retention time, pond volume, morphology and area, outlet location (surface, bottom) and many others (Adámek et al., 2014). In general, water discharged from ponds supplied with good-quality water has a higher temperature, and increased nutrient loading (mainly in the form of ammonia and phosphates), dissolved substances and suspended solids content (Kanclerz 2005; Kopp et al., 2012; Všetičková et al., 2012). On the other hand, nitrate concentrations are usually lower due to water retention. Changes in biochemical oxygen demand (BOD) and chemical oxygen demand (COD) depend primarily on inflow water quality, the outflow having reduced BOD and COD when inflows have high organic substance loading (Svoboda and Koubek 1990; Masseret et al., 1998; Všetičková et al., 2012). On the contrary, inflow water poor in organic content will become enriched in the pond, and consequently, outflows will have increased BOD and COD (Všetičková et al., 2012). Elevated levels of BOD and COD, along with increased suspended solid and phosphorus (P) concentrations and decreased oxygen content, are typically associated with pond draining during harvesting.

Water retention in a pond will affect the oxygen regime, with a decline in dissolved oxygen (DO) concentration due mainly to respiration of water organisms and consumption of oxygen via decomposition of organic substances (including unused feed) and oxidation of inorganic substances. On the other hand, pond water can be saturated with oxygen from phytoplanktonic photosynthesis during the day. Supplementary feeding of pond fish requires a high input of organic matter and nutrients into the ecosystem; ponds with high fish stock densities, therefore, are particularly prone to low DO connected with high organic waste and feed levels. Excess organic material consumes oxygen during decomposition and can drive DO concentrations in ponds to dangerously low levels should sudden destratification occur. In addition, nutrients derived from organic waste and unused feed can enhance algal growth: blooms of planktonic algae resulting in extremely high DO concentrations during daylight and super-saturation of oxygen in the epilimnion. Intensive ecosystem respiration, which can consume substantial quantities of oxygen at night, may result in anoxic conditions in the predawn hours (Schroeder 1974; Boyd 1982; Jana and Sarkar 2005).

According to a number of studies, the negative impacts of pond aquaculture on the environment can be summarized as (in particular):

1. Modification of water temperature and flow rate profiles (Billard and Perchec 1993, Beveridge 1984, Všetičková et al., 2012).
2. Increased concentration of suspended solids, BOD, COD, forms of N (including ammonia) and phosphorus (Warrer-Hansen 1982, Muir 1982, Boyd and Tucker 1998, Kanclerz 2005, Petrovici et al., 2010, Kopp et al., 2012, Všetičková et al., 2012).
3. Reduced concentration of dissolved oxygen (Bergheim and Silvertsen 1981, Boyd and Tucker 1998, Všetičková et al., 2012).
4. Alteration of water quality due to the use of chemicals and antibiotics (Buchanan 1990, Boyd and Massaut 1999).
5. Generation of organic-rich sediments (Holmer 1992, Lin et al., 1998, 1999, Lin and Yi 2003, Vallod and Sarrazin 2010).
6. Occurrence of algal blooms in eutrophic waters (Gowen et al., 1990, Potužák et al., 2007, Jahan et al., 2010).
7. Modification of the biotic index (based on invertebrate communities) and of the

index of biotic integrity (based on fish populations) (Gowen et al., 1988, Trigal et al., 2009, Petrovici et al., 2010).
8. Genetic pollution and escape of undesirable and invasive fishes (e.g. *Carassius gibelio* and *Pseudorasbora parva*) (Cross 1992, Kalous et al., 2004, Gozlan et al., 2005, Tsoumani et al., 2006, Musil et al., 2007, 2010, Lusk et al., 2010).
9. Increased risk of disease spread (Hepher and Pruginin 1981, Hubbert 1983, Akoll et al., 2012, Hoverman et al., 2012).

4.3. Conclusions and knowledge gaps

The EC regulations regarding environmental impact and interactions in relation to the sea bottom are not very specific.
Norwegian rules for conventional farming state that the allowed biomass on a location is based on the recipient capacity to handle organic load. The farmers have to document the status of the sea bottom annually by undertaking third party NS 9410 inspections. Minimising the organic load from the farms include reducing feed waste and faecal material, there is little knowledge regarding the amount of lost feed but it has been assumed to be as high as 5%.
With regard to wild fish feeding there are no EC regulations that apply specifically. Attraction of wild fish to open cage farms is a global phenomenon, and more than 160 species belonging to about 60 families have been detected in the near vicinity of such farms.
Marine fish farms attract wild fish by providing uneaten fish feed, structural habitats and by attracting small prey species. The more ecological consequences of attracting wild fish, and wild fish feeding on waste feed may be regarded as environmental effects, at least in a wider definition; it is well known that farm aggregated species, will achieve different liver size, and different lipid content and fatty acid composition.The amount and composition of fatty acids also affect the quality of the offspring.

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6. Occurrence of algal blooms in eutrophic waters.
7. Modification of the biotic index (based on invertebrate communities) and of the
index of biotic integrity (based on fish populations).
8. Genetic pollution and escape of undesirable and invasive fishes (e.g. Carassius gibelio and Pseudorasbora parva).
9. Increased risk of disease spread.

5. State of the art on recycling and waste

5.1. Current regulations

834/2007 Article 5(c): *In addition to the overall principles set out in Article 4, organic farming shall be based on the following specific principles: (c) the recycling of wastes and by-products of plant and animal origin as input in plant and livestock production;*

No 710/2009 Article 6b 5: *Aquaculture and seaweed business operators shall by preference use renewable energy sources and re-cycle materials and shall draw up as part of the sustainable management plan a waste reduction schedule to be put in place at the commencement of operations. Where possible, the use of residual heat shall be limited to energy from renewable sources.*

No 710/2009 Article 6d 4: *Ropes and other equipment used for growing seaweed shall be re-used or recycled where possible.* (see chapter 3 Production system, paragraph 9).

5.2. Current scientific knowledge

5.2.1.Marine production

The overall feed conversion ratio (FCR) in Norwegian salmon farming in 2010 was 1.3 (Ytrestøyl et al., 2011), meaning that 1.3 tons of feed (with dry matter content of approximately 95 %) was used for each ton of salmon produced, all losses in fish and feed production included. Salmon feed has high nutrient density, and feed spill and undigested material result in loss of significant amounts of nutrients and energy. Whereas salmon feed earlier was based on mainly fish meal and fish oil, today's feed contain an increasing amount of plant ingredients (Ytrestøyl et al., 2011), which again result in an increasing amount of indigestible fiber and thus waste. The apparent digestibility of the dry matter of salmon feeds of commercial type for non-organic farming from 2011 was approximately 65 % (Oehme et al., 2013). Thus, 35 % of the dry matter of feed was lost as undigested material, which, with a FCR of 1.3, corresponds to approximately 450 kg of dry matter from faeces per ton of salmon produced. With regard to waste production, feeds based largely on ingredients animal origin may be desirable. Due to the raw material situation however, considerable amounts of plant ingredients may be necessary in feeds for organic salmon farming if the organic farming shall achieve a fair share of the market.

The feed spill in commercial salmon farming is difficult to estimate, but 7 % has been suggested (Gjøsæter et al., 2008). With an FCR of 1.3, 7 % feed spill amounts to 91 kg of feed loss per ton salmon production.

Waste from farmed fish may affect the environment locally (Husa et al., 2010) compared to waste from wild fish which are distributed over large areas. Waste from salmon farms located at sites with sufficient water current and water exchange is not assumed to affect the coastal environment negatively (FKD, 2009). As long as the salmon farm has a suitable location, the main concern about the wastes is therefore the loss of valuable nutrients. This is particularly crucial for phosphorus (P), which is a limited resource and an essential element for all plants and animals. The high inclusion of plant ingredients in fish feeds causes a flow of P from land to sea. Collection and recycling of the wasted P is obviously desirable.

The waste that settles from salmon farming and form a sludge that can be mechanically collected, consists mainly of faeces and feed spill. Faeces have a completely different composition than that of feed, with a high concentration of indigestible fiber and minerals, and with a high salt content when the fish is reared in sea water.

According to the figures above, approximately 0.54 tons of dry matter from faeces and feed is lost per ton of salmon produced. Sludge from land based aquaculture has low dry matter content, in the range 1-10 % (Chen et al., 1993; Gebauer and Eikebrokk, 2006), whereas 16 % dry matter was reported for a sludge that had settled on the bottom below sea cages (Teuber et al., 2005). Assuming 10% dry matter in sludge, 0.54 ton dry matter would correspond to 5.4 tons of sludge per ton salmon production. However, dry matter content in sludge is highly variable. Besides, due to some of the waste being dissolved or dispersed in the water, the amount of collectable dry matter is well below 0.54 ton per ton salmon produced. The fraction that dissolves or disperses can be estimated (Chen et al., 1997), but will vary largely depending on factors such as feed properties. Therefore, the amount of sludge formed per ton salmon produced can not be predicted from these estimates.

The technology for collecting sludge from open sea cages is poorly developed, whereas in land based aquaculture (flow-through and recirculation systems) and potentially in the novel closed sea cages, waste particles larger than a certain particle size can be collected with various filtering methods and potentially recycled (Cripps and Bergheim, 2000; Gelfand et al., 2003; Rosten et al., 2013; Sharrer et al., 2010; Summerfelt et al., 1997; Tal et al., 2009).

One main challenge is the low dry matter content and thus large volume of sludge. To avoid transport of large volumes of water, recycling of nutrients thus requires technology for effective reduction of water, and/or reuse of the sludge at or near the fish farming site. At present, technology for further water removal of the sludge is being developed. For land-based salmon farming, it is realistic to expect that technology that allows almost complete removal of water from sludge is developed within a couple of years. Dry waste can be transported for optimal re-use of nutrients.

Several possible ways for recycling nutrients from sludge are being investigated. The simplest solution, provided the waste is from fresh water aquaculture, is to spread the collected sludge directly on farm land as a fertilizer. Due to transport of large volumes, this is limited to agriculture fields within a short distance from the fish farming site, and odor can be a severe problem. The heavy metal concentrations of the sludge must be within regulations for use as an agricultural fertilizer, and the risk of pathogens needs to be considered. Furthermore, unbalanced plant nutrient ratio can reduce growth in the agricultural crop (Brod et al., 2014). However, aquaculture waste has shown potential as agricultural fertilizer, even sludge from salt water aquaculture, particularly when mixed with other wastes or components (Brod et al., 2012; Brod et al., 2014; Teuber et al., 2005). This may be the simplest way of recycling aquaculture waste if the logistic challenges (volume, transport distance and sufficient recipient areas) are solved.

Aquaculture sludge is extremely susceptible to putrefaction, which produces malodorous, toxic, explosive gases. Sludge can be stabilized by alkalization, which prevents decomposition and makes the sludge more hygiene (Bergheim et al., 1998).

Sludge from freshwater aquaculture can also be stabilized by wet land (Summerfelt et al., 1999) and composting (Chen et al., 1997; Marsh et al., 2005). For both these options however, the large volume of sludge is a challenge, and the storage of sludge must not be in conflict with the legislation.

Aquaculture sludge has been used as medium for growing microalgae, which were harvested and processed to a meal intended to be a feed ingredient (Dickson, 1987). The quality and prize could however not compete with other feed ingredients on the market. Growing polychaeta from the sludge may have a larger potential as feed ingredient (Palmer et al., 2014). Technology for large scale polychaeta production is however not available at present.

Aquaculture sludge contains large amounts of energy. Assuming a FCR of 1.3 in salmon farming, and energy content of 25 GJ/ton in the feed (Ytrestøyl et al., 2011), 7 % feed spill alone will amount to 2.3 GJ of energy loss per ton salmon produced. Energy from sludge can be captured by anaerobic digestion which produces biogas. A considerable amount of research on production of biogas from aquaculture sludge has been performed (Gebauer, 2004; Gebauer and Eikebrokk, 2006; Mirzoyan et al., 2012; Mirzoyan et al., 2010; Tal et al., 2009). However, this is still on the experimental stage, and satisfying technology for large scale biogas production from aquaculture sludge is still not developed. Biogas production is labor demanding, high fat content from spill feed can be a challenge for the methane producing bacteria, and biogas production does only result in limited volume reduction. With present technology, sludge from one land-based fish farm is not sufficient to run a viable biogas reactor, and effective de-watering technology is required for long distance transport to central reactors. Biogas production leaves a digestate rich in nutrients, which has a potential as a soil fertilizer (Haraldsen et al., 2011).

Fish also excrete nutrients directly to the water which are not captured in the sludge. Nitrogen (N) and other elements/substances are excreted from kidney, gills and skin. The non-faecal loss of N in salmon depends on factors such as feed intake, feed type and fish size, but in one trial the non-faecal N-loss corresponded to 23.8 kg per ton salmon produced (Aas et al., 2006).

Non-faecal excretions are dissolved in the water. The amount of material from faeces and feed spill that is dissolved or dispersed in the water is not known, and will vary among feeds. The total amount of nutrients transferred to the water per ton salmon production can therefore not be calculated. Dissolved and dispersed waste can be captured by growing species that extract the dissolved nutrients from the water, such as macro-algae (kelps), filter feeders (e.g. blue mussel) and deposit-feeders (e.g. sea cucumber). In such polyculture (integrated multi-trophic aquaculture, IMTA), species such as kelp and blue mussel grown nearby a salmon farm has shown variable, but generally promising results (Broch et al., 2013; Chopin and Robinson, 2006; Handå et al., 2013; Handå et al., 2012; Irisarri et al., 2013; Lander et al., 2013; MacDonald et al., 2011; Molloy et al., 2011; Nelson et al., 2012; Reid et al., 2010; Reid et al., 2013; Ridler et al., 2007; Troell et al., 1997; Troell et al., 2009; Wang et al., 2013).

IMTA is currently at a developing stage, and the economy in this way of farming is still unclear (Ridler et al., 2007). Besides, for IMTA to be a viable solution there has to be a market for the end products (e.g. kelp and blue mussels), and energy cost of processing and transporting these must be considered. There are also biological challenges, such as utilization/retention efficiency of salmon waste by the other IMTA-species, match/mismatch of growth cycle of these species and that of salmon's maximum waste production, optimizing scale of the production of each species, and production of faeces from blue mussels which require a certain efficiency in waste absorption to achieve net removal of salmon waste (Handå et al., 2013; Irisarri et al., 2013; Reid et al., 2010; Troell et al., 2003). Furthermore, growing blue mussels near a salmon farm may increase the biofouling of the net cages.

Blue mussels of high quality can be used for human consumption, and mussels of lower quality have a potential as an ingredient in salmon feed (Troell et al., 1997). However, the shell of the mussel represents a large portion of the produced volume. Several ways of utilizing clam shells have been examined (ÁLvarez et al., 2012; Barros et al., 2009; Kwon et al., 2004; Yang et al., 2005; Yoon et al., 2003), and a solution for utilization of this by product is necessary for large scale IMTA with blue mussels to be viable.

5.2.2. Fresh water production; carp pond culture

In comparison with the existing voluminous literature on trout farm effluent and its impact on the environment, the characteristics of carp pond effluents are poorly documented (Hlaváč et al., 2014). This is probably due to the fact that carp are mainly produced in extensive, semi-intensive or integrated systems on a worldwide basis (Kestemont 1995; Woynarovich et al., 2011). Such ‘‘traditional’’ systems are generally considered non-polluting and acting as stabilizing elements in the ecosystem (Manz et al., 1988; Szücs et al., 2007; Barszczewski and Kaca 2012). Pond may also contribute to the self-purification processes in surface waters (Lewkowicz 1996). Research carried out in Austria by Kainz (1985), for example, found no negative impact on receiving water quality from traditionally managed carp pond facility. As indicated by Butz (1988), Banas et al. (2002, 2008) and Vallod and Sarrazin (2010), however, nutrient outflow may not pose problems during the growing season, but pond draining preceding the annual harvest and during harvesting operations can significantly increase loading in receiving waters due to mobilisation of sediments.

Pond aquaculture waste

The influence of supplementary fish feed on discharged water quality will depend on the composition and physical characteristics of the feed used, the technology used in its production, its digestibility, palatability, quality of the components supplied, and feeding technique. The waste produced can be divided into either solid or dissolved phase. Solid waste, consisting of settleable and suspended solids, mainly originates from uneaten and/or spilled feed and from excreted faeces. Part of the dissolved waste (i.e. organic substances, ammonia) originates from metabolites excreted by fish through the gills and in urine, the rest originating from disintegration/resuspension of nutrients from both the settleable and suspended solid waste fractions (Amirkolaie 2011; Adámek and Maršálek 2013). In the carp pond, the mud is mixed with water by carp in its search for food in the bottom (Hepher 1958). In principle, supplementary feeding intensity in semi-intensive carp ponds is adjusted according to quantitative and qualitative measures of zooplankton development, which is ultimately controlled by fish predation (Schlott et al., 2011). In intensive aquacultural systems, between 20 and 40 % of dietary dry matter is incorporated into the fish body and the remaining part excreted (Siddiqui and Al-Harbi 1999; Verdegem et al., 1999; Brune et al., 2003). When feeding fish with cereals alone, the fraction of nutrient supply driving the bacterial–detrital food chain can rise to almost 95 %, with only 5 % directly utilised for fish growth (Olah 1986). The amount of faecal waste produced can range between 0.2 and 0.5 kg dry matter per kg feed, depending on feed composition, fish species and temperature (Chen et al., 1997). Whichever system is used, the proportion of uneaten or spilled feed will range between 5 and 15 % (Beveridge et al., 1997; Cho and Bureau 1997). In order to improve this situation, a number of studies have been undertaken that address fishpond management and feeding strategy, investigating such issues as new fish feed mixtures with lower nutrient content and improved utilisation of the feed supplied (Adámek et al., 1997).

In all aquacultural systems, waste is partially discharged with effluent water. Both the amount and composition of waste discharged, however, will differ depending on the system employed. In pond systems, the vast majority of total waste remains in the system, with part of the organic waste mineralised in situ (Fig. 1; Verdegem et al., 2001). For this reason, carp ponds can be of great ecological value, though sustainability can only be maintained if stocked at production intensities of at least 500–1,000 kg ha-1, thereby guaranteeing minimum profit without obvious signs of pond degradation (Knösche et al., 1998; Knösche et al., 2000). In addition, ponds may trap nutrients from the outer catchment area and transform them through primary and subsequent production links within the pond food chain (Füllner et al., 2000). Many critics, however, point out that carp culture pollutes the environment as effluent water enters downstream watercourses (Petrovici et al., 2010).

In intensive aquaculture, most dissolved nitrogen (N) and P come from metabolic waste products excreted by fish (Hakanson et al., 1998; Lemarie et al., 1998; Gondwe et al., 2011), with levels of N and P in fish food (Table 2) and its efficiency of use influencing the amount excreted (Rodehutscord et al., 1994). Reducing dissolved N and P output is now considered a key element for long-term sustainability of aquaculture around the world (Phillips et al. 1993; Cho and Bureau 1997; Sugiura et al., 2006). Fish can retain 20–50% of feed N and 15–56% of feed P (Schneider et al., 2004; Rahman et al., 2008b; Adhikari et al., 2012), releasing the remainder into the water where it can then be converted to valuable products by phototrophic and heterotrophic organisms (Schneider 2006). The use of balanced diets, therefore, can significantly reduce the amount of these compounds in the water (Hasan 2001), with rearing size, fish species, rearing practice, alimentary handling and food characteristics also affecting the amount of alimentary residue (Mallekh et al., 1999). The use of extruded diets has proved to be an important advancement in fish nutrition. These possess higher stability and digestibility, resulting in a significant reduction in the amount of nutrients excreted into the rearing water (Johnsen et al., 1993). As feeding efficiency improves, the waste N and P concentrations decrease, and thus, ammonia concentration is often seen as the limiting water quality parameter in intensive aquaculture production systems (Thomas and Piedrahita 1998).

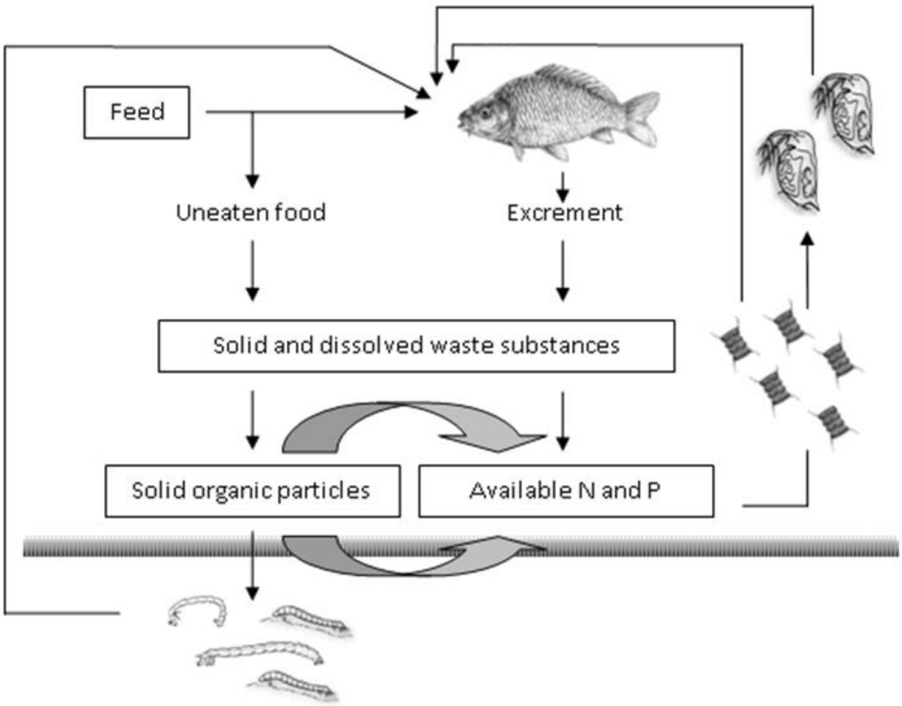

Fig. 1 The fate of feed in a semi-intensively managed fishpond (Rahman 2006; Adámek et al., 2014)

Nitrogen

Fish are able to utilise protein very efficiently, despite using a significant proportion of digestible protein for energetic purposes (Kaushik et al., 1982; Kaushik and Dabrowski 1983) and producing large amounts of nitrogenous metabolites (Dosdat et al., 1996). Results from a variety of culture systems indicate that, on average, about 25% of N (ranging from 11–36 %; Fig. 2) supplied in feed or other nutrient input is retained by the target organism (Avnimelech and Lacher 1979; Hargreaves 1998; Rahman et al., 2008a;
Nowosad et al., 2013). Little information is available regarding the effects of N input and output on N dynamics in aquacultural ponds (Gross et al., 2000; Gál et al., 2003, 2013), and a more complete understanding of the factors regulating ammonia and nitrite concentrations and the exchange of nitrogenous compounds between sediment and water is needed (Hargreaves 1998). Uneaten feed and faeces, however, do contribute to the system's organic loading. Microbial decomposition of organic matter in the water and sediment (see Crab et al., 2007) leads to increased levels of ammonium (Read and Fernandes 2003), which in turn may be transformed into nitrite, nitrate and gaseous N (though formation of N gas is considered negligible in aquaculture ponds). Data on the amount of ammonia excreted by fed and starved fish as well as the dynamics of ammonia excretion by fish of different size are limited (Nowosad et al., 2013). Both ammonium and nitrite are harmful to fish, even at low concentrations. Regarding ammonium in water, it is in equilibrium with ammonia, depending on pH and temperature (Timmons et al., 2002). It would appear that ammonium is at least two orders of magnitude less toxic to the fish than ammonia, being toxic for commercial fish at concentration above 1.5 mg N l^{-1} (Eshchar et al., 2006).

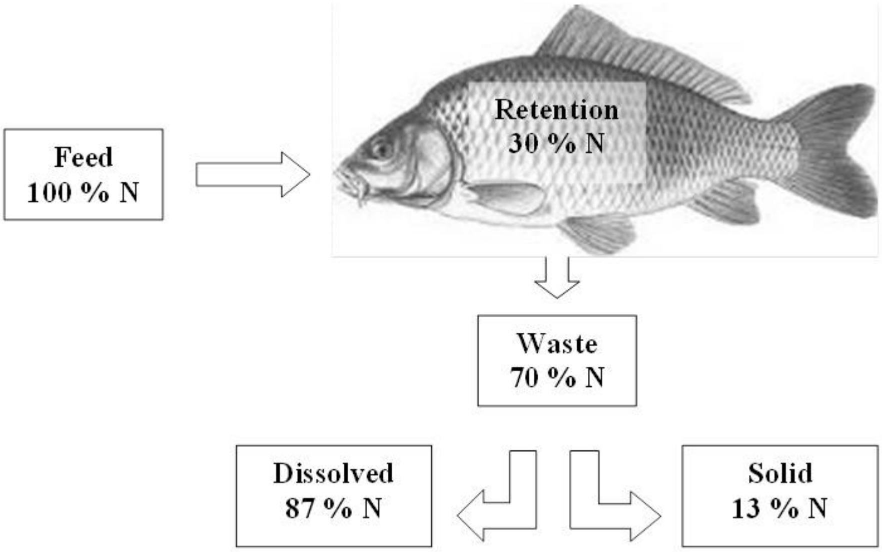

Fig. 2 Nitrogen retention in fish (adapted from Jirásek et al., 2005)

Ammonia can also cause a decrease in growth and feed utilisation in different fish species (Biswas et al., 2006). On the other hand, nitrite can cause severe damage in fish, even mortality at 0.43 mg l^{-1} (Koltai et al., 2002), mainly through the decrease in oxygencarrying capacity of the blood and causing anoxic conditions in organs and tissues (Kroupova et al., 2005). However, the toxic effects of nitrite and ammonia depend on a large number of external and internal factors (Svobodová et al., 2005; Colt 2006).

The inherent nutrient utilisation efficiency of fish implies that N loading in fishponds may be limited by the pond capacity to assimilate nitrogenous excreta (Hargreaves 1998; Paspatis et al., 2000). Factors affecting N excretion include fish species and time after food intake (Lupatsch and Kissil 1998), while an excess of amino acids in feed results in amino acid catabolism, which is associated with ammonia excretion and a loss of energy (Lloyd et al., 1978). In addition to protein content of feed, the balance between digestible protein and digestible energy in the diet can result in an increase in N retention efficiency and a decrease in ammonium waste excreted (Kaushik 1994, 1998; McGoogan and Gatlin 2000).

The use of non-protein energy sources such as fat or carbohydrate, to meet energy requirements, can improve protein retention (Keshavanath et al., 2002), thereby also reducing ammonium waste. This phenomenon is commonly called the ‘‘protein-sparing effect’’ and has been demonstrated in a number of species (Kaushik 1998). In semiintensive carp pond culture, ammonia and urea excretion by fish is of less importance as, under favourable conditions, both compounds are immediately incorporated in the pond ecosystem’s ‘‘metabolism’’.

Vegetable protein, the primary protein source in carp ponds, affects feed utilisation and N waste differently according to its origin. Generally, vegetable protein has a poor amino acid balance, which reduces N retention and, consequently, increases N excretion. In common carp, total N loading calculated based on whole-body N retention is 31–86 kg N per 1 tonne of fish produced (Jahan et al., 2002). Thus, alternative protein sources, such as fish meal and soya bean meal, have been suggested in order to improve N assimilation and utilisation efficiency (Hargreaves 1998).

However, ponds have a large nitrogen retention capacity due to denitrification and fixation in sediments (El Samra and Olah 1979; Olah et al., 1983; Pokorný et al., 1999). Several data for nutrient budget of freshwater fishponds operated under various climatic conditions (Avnimelech and Lacher 1979; Boyd 1985; Foy and Rosell 1991) and carpbased ponds in the temperate zone (Olah et al., 1994; Schreckenbach et al., 1999; Knösche et al., 2000) have been reported. According to Knösche et al. (2000), fishponds retain on average 78.5 kg N ha^{-1} $year^{-1}$. However, nitrogen retention of 93 kg N ha^{-1} $year^{-1}$ was reported by Olah et al. (1994), as a result of an analysis of nitrogen input and output data for a 20-year period. The average nitrogen retention in Germany and Hungary was 43 kg ha^{-1} $year^{-1}$ in a number of fishponds (Schreckenbach et al., 1999).

Phosphorus

While fish can absorb P from water, dietary supplementation is usually necessary due to low waterborne P concentrations (Phillips et al., 1958). Under traditional commercial aquacultural feeding regimes, retention of dietary P is around 20%, the rest (68–86 %) being excreted (Avnimelech and Lacher 1979; Crab et al., 2007; Lazzari and Baldisserotto 2008; Rahman et al., 2008a). Excess P in fish diet results in higher levels of excreted P, and this is one of the main causes of eutrophication in ponds (Kim et al., 1998; Jahan et al., 2003), often also resulting in impaired water quality downstream. Sediment-bound P is a major problem in carp ponds, and about half the P in a pond can be controlled by monitoring the output of solids (Pursiainen 1988; Vallod and Sarrazin 2010).

With the global concern for reducing water pollution, it is becoming imperative that the fish food industry reduces P excretion in fish (Rodehutscord et al., 2000). One way of addressing this is in the formulation of new fish feeds that produce less P pollution while maintaining adequate levels of available P to support growth (Jahan et al., 2003; Satoh et al., 2003; Bueno et al., 2012). The bioavailability of P from fish meal is lower for carp than rainbow trout *Oncorhynchus mykiss* such differences probably originating from the lack of gastric digestion in stomach-less carp (Lall 1991; Satoh 1991; Jahan et al., 2001). Feed composition, therefore, has a great impact on P digestibility, retention and loss (Amirkolaie 2005a).

The level of unretained P is largely a function of the amount of P in the feed and its bioavailability (Buyukates et al., 2000). In general, P is found in all plant and animal components used in formulate feeds; its bioavailability varies greatly, however, depending on the particular component (Table 3). Plant protein ingredients, such as corn gluten or soya bean meal, have a lower P content compared to fish meal or other animal byproducts: a desirable characteristic for low-polluting diet formulation (Cho et al., 1994; Sarker et al., 2011; Yang et al., 2011). Vegetable sources, however, generally possess larger amounts of phytate form P, a form unavailable to fish as they do not possess the enzyme phytase (NRC 1993; Cho and Bureau 2001; Kumar et al., 2012). As a consequence, most phytate-P ends up being excreted into the water and may cause algal bloom pollution (Baruah et al., 2004). The use of high-protein ingredients with a high percentage of digestible P, therefore, should help to reduce the concentration of unavailable P in feed (Cho et al., 1994). In common carp, total

P loading calculated based on whole-body P retention is 8.9–26.4 kg P per 1 T of fish produced (Watanabe et al., 1999; Jahan et al., 2000, 2001, 2002).

Reduction of carp pond waste discharge into the environment

Feed quality improvement

Ingredient digestibility and nutrient composition are among the main factors affecting total waste output in aquacultural production, and therefore, efforts at minimising further waste discharge from aquaculture should aim to improve diet formulation and processing. Solid waste in aquaculture is mainly composed of undigested starch and fibre from grain and plant ingredients: undigested protein and fat being low in solid waste as they are highly digestible (Cho and Bureau 2001). Application of highly digestible feed, however, cannot solve the issue of faeces production completely as digestion in fish is naturally limited and a certain fraction of the feed will always remain undigested and excreted in faeces (Cho et al., 1994). Furthermore, as availability of fish meal and fish oil becomes limited in future (Hardy 1996; Gatlin et al., 2007; Amirkolaie 2011), modern farming systems for herbivorous, omnivorous and carnivorous fish are all expected to rely more on supplementary diets containing a high percentage of plant ingredient (Naylor et al., 2000; Hardy 2008; Hua and Bureau 2012).

The composition of feed and the way it has been processed can alter the physical properties of faeces, thereby influencing the efficiency of solid waste sedimentation (Amirkolaie et al., 2005b). Starch is a cheap source of energy, and its inclusion in fish feed can influence faeces stability (Han et al., 1996; Brinker and Friedrich 2012). Stable faeces have a larger particle size, settle more quickly and are more efficiently incorporated into decomposition and bioturbation processes at the pond bottom. Plant ingredients always contain a fraction of starch and the addition of starch to an aqua diet can reduce the dissolved nitrogenous waste of many fish species by increasing the non-protein dietary energy content (Steffens et al., 1999; McGoogan and Gatlin 2000).

Over the past few decades, there have been many changes in feeding and feed technology aimed at reducing the production of solid waste through uneaten or spilled feed (Bergheim and Asgard 1996), including technological treatments (e.g. extrusion and expansion) that have improved the physical characteristics (e.g. water stability, leaching) of fish feeds (Wilson 1994; Misra et al., 2002). The feeding habits of benthivorous fish (such as carp) are of special importance in this context as regards their role in ingestion of feed sinking to the bottom. In searching for spilled feed, they release large quantities of nutrients into the water column, thus enhancing phytoplankton production (Adámek and Maršálek 2013). This may be especially important in aquacultural carp ponds (Avnimelech et al., 1999), particularly in those with older carp that receive supplementary food from the onset of the growing season (Kloskowski 2011).

5.3. Conclusions and knowledge gaps

At present, knowledge and technology for a near complete recycling of nutrients from salmon farming is not developed. Altering the regulations is therefore not recommended. However, solutions for collection, de-watering and re-use of waste are presently being sought for in non-organic salmon farming. The technology is therefore expected to be improved during the next years and thus there may be a basis for reconsidering the regulations for organic salmon farming within near future.

6. Ethical aspects related to potential environmental impacts and interactions

All through these reviews *feed composition* is considered important from an environmental point of view, whereby the production steps and transport of feed are described as the main concern. It is stated that other factors and elements are excluded from LCAs, leading to an incomplete picture of the situation.

Feed for omnivorous fish (such as Atlantic salmon, the most frequent example in the reviews) should stem from organic aquaculture, fishmeal and fish oil from organic aquaculture trimmings, from trimmings from fish caught for human consumption in sustainable fisheries. I.e. catch of wild fish for feed production is not allowed. This is important, as conscious consumers are aware of the negative effects of global industrial fisheries on fish stocks and on biodiversity. Hence, the development of alternatives, e.g. using trimmings, is essential to stay sustainable and to meet the basic principles of health and ecology.

In organic finfish farming of omnivorous species a maximum of 60% vegetable feed is allowed, but such a high portion increase the risk of high levels of fossil energy use, nutrients leakage and nitrogen fertilizer etc. in the agrarian production chain. Considering the feed components, soy should be GMO free in organic farming, and this is available in Brazil, a positive matter which on the other hand seems to be ‘countered’ by higher level of CO_2 emissions than production in e.g. Canada and the U.S. Further, production of oil from rape seeds and palm oil are regarded more energy and water efficient than camelina oil, and rape seed less sustainable than palm oil – which on the other hand is said to contribute to deforestation of rain forest.

Hence, there seems to be no perfect feed, and the overarching task will be to create a prioritizing of which factors are most pressing, and an act of balancing of the consequences in terms of – at least - sustainability, fish welfare and consumer confidence.

One way to do so is of course to reconsider the entire concept /market from an ‘organic principles perspective’ – can farming of carnivorous fish species at al sustainable? Farming of omnivorous species would probably be more sustainable, and easy to defend to conscious consumers, as would pure Given the fact that some stakeholders (at the first OrAqua stakeholder meeting in Istanbul in 2014) expressed a concern that the difference between conventional and organic is hard to detect, a shift to accepting farming of only herbivorous fish, e.g. carp, tilapia or catfish, and shellfish might be worth considering.

Environmental aspects in the reviews on the latter, sea food except for fish, seems mainly to mention the positive effects on water quality and the fact that farming does not cause disturbances on the sea bottom, as does wild harvesting. There is also a possible use of bi-products (shells) in other agrarian production (chickens and layers). Hence, taken the above mentioned aspects of omnivorous or carnivorous fish species into account, organic shell fish seems to fulfil at least three of the organic, ethical principles such as Health, Ecology and Care. There is probably potential to also meet the principle of Fairness, depending on how the market structure is developed. Given the industrialised situation of aquaculture and fishery in general, incentives to help small organic farmers develop sea food farming are one way to meet also the principle of Fairness.

Considering the theme of sea food as a way to avoid harm to the sea bottom, another issue elaborated on in several reviews is *feed waste* from fish cages/containments. The estimated loss is between 5% (review Sea bottom and Wild fish feeding) to 35% (review Recycling and waste) of distributed feed, hence - it seems difficult to estimate the waste as it varies largely between feeding regimes. This is of course also of ethical relevance, as choice of improvements is to be formulated on a background on plausible descriptions of reality. When the fact part is limited, so are the aspects to take into ethical account.

Relevant factors in this 'open end' issue are e.g. the location of the cage/farm system in relation to water flow, kind of feed, the consistency of the feed and the waste itself. As large amounts of waste leads to large amounts of sludge, which so far not possible to use for biogas or else, it can hardly be considered as anything else than an environmental cost.

As to the content and connected digestibility the situation seems to be parallel to the difficulties in choice between CO_2 emission and GMO above. According to the review of the potential environmental impacts and interactions in relation to sea bottom and wild fish feeding, a higher amount of plants reduces digestibility and mechanical stability of the faecal material, leading to a higher frequency of smaller parts, which in turn reduces the local sedimentation. According to another review (energy use and CO_2 released during production of Atlantic salmon) the high portion of plants leads to higher footprints, as the feed efficiency is lower compared to feed based on trimmings or whole fish.

Hence, there is an ethical balance to be undertaken between – at least – the following factors: waste composition, amount of waste, and its consequences on the sea bottom, and the foot print in the production of the feed. In addition, such a balance would need to take biodiversity in relation to wild catch, as well as animal welfare and health for the farmed fish into account.

There are also further issues related to *farmed fish and its relation to other species, and its own*. This is mirrored both in the issue on escapes, and in the review on land based systems. As to the former, the question is whether or not to it is possible to recapture (i.e. catch and use/kill, not return to the cage?) and mainly how to best prevent escapes in order not to influence the local ecosystem. It is suggested that measures – above preventive management of potential weather damages - need to be e.g. species –specific, that damage by biting on the cages might be due to hunger, and that enriched environments reduces the incentives to escape. Hence welfare concerns are presented as measurements so to prevent, escapes which in turn should be avoided to protect biodiversity. This approach might be seen as a 'typical' organic, i.e. holistic, way of reacting which has potential to meet consumer appreciation.

Concerning the latter, other species as related to land based farming systems, there is only a hint in the review on hindrances on mobility for wild fish when a farm is occupying large part of a water system. One response to such to environmental and biodiversity concerns has been to develop closed systems. As they need more of technical systems and solutions, the critique has been that they are not 'natural enough'. As soon as such an argument is used, one should be cautious of the definitions or intended meaning, as it could refer either to 'according to nature', 'not created by humans' or just 'not an artefact'. Independently of choice of definition, it is difficult to argue that fish farming, on the whole, is a 'natural' undertaking: large amounts of fish are bred, selected, fed and kept in a small enclosure hindering most of their species-specific behaviours, and slaughtered mechanically – all steps developed by humans. Hence, there need to be another argument against recirculation systems being unsuitable for organic farming. One candidate could be linked with a strive to reduce the size or number of fish in each enclosure, in order to ensure species-specific behaviour and reduce the percentage of escapes: in a less intensive system, less technical solutions are needed. However, if for ex aeration is needed to ensure good enough water quality, the principle of Care would apply.

Due to the early stage of the project, an overarching elaboration on the question of how to create a global sustainable food production – though vegetables, sea food, fish or mammalian meat - will not be considered here, but fits in the last phase of the project.

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