

Extracted and integrated/synthesized information from WP2 and WP3 (1st stakeholder event)

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Responsible for Deliverable: Alfred Jokumsen, DTU

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0.0 Preface

Based on the analysis of the preliminary information provided by WP 2 and WP 3 and feedback from the 1st Stakeholder event in Istanbul 11th-12th October 2014 this document is an extract and synthesis of this information on key issues related to the current regulation on organic aquaculture including recommendations and research gaps to be considered to underpin future growth of the European aquaculture sector.

However, it is noticed that the reviewing and data collection is still on-going, e.g. WP 3 is currently analysing both literature and making a survey together with an economic model and analysis of institutional frameworks. The deliverables from WP 2 (D2.1-2.2) and from WP 3 (D3.1 – 3.3) due in Month 18 will provide final reviews of production related issues as well as consumer and socio-economic issues and institutional frameworks. Hence, this deliverable 4.1 provide extracted and synthesized information so far from WP 2 and WP 3 integrated with information and feed-back from the 1st stakeholder event.

The report is structured in the following way:

Section 1 gives the objectives of this deliverable (D4.1).

Section 2 gives a summary including the Power Point presentation: “Presentation of the synthesis of the scientific review process” presented at the 1st stakeholder event in Istanbul.

Section 3 summarizes the feed-back from the 1st stakeholder event in Istanbul 11th – 12th October 2014.

In section 4 the overall conclusions including recommendations and research gaps are summarized.

In the Appendix section A1 – A5 specific key issues related to the regulation are preliminary reviewed to extract and synthesize key information and to identify recommendations and research gaps for each subject.

Finally a reference list is given in section A6.

1.0 Objectives

The objectives of this document is to present an analysis of the preliminary information provided so far by WP 2 and WP 3 and feed-back from the 1st Stakeholder event in Istanbul 11th-12th October 2014. However, the reviewing and data collection, handling and interpretation is still on-going and final reviews will be provided by Month 18.

Based on the current EU Regulation on Organic Aquaculture the report covers a range of challenging issues for consideration in revision of the regulation. This includes Nutrition, Welfare, Health, Veterinary treatments, Biosecurity, Productions systems, Potential Environmental Impacts and Interactions, Consumer aspects, Socio-economy and Institutional Frameworks.

2.0 Summary

The analysis of the preliminary information provided by WP 2 and WP 3 and feed-back from the 1st Stakeholder event in Istanbul 11th -12th October 2014 are summarized in the following key issues related to the current regulation on organic aquaculture. Inserted at the end of the summary is the presentation: “Presentation of the synthesis of the scientific review process” presented at the 1st stakeholder event in Istanbul.

In consideration of fish health, product quality and low environmental impact a general concern was expressed about the intended **sourcing of feed ingredients** for feed for carnivorous fish (EU Reg. 710/2009 art. 25k). To achieve nutrient balanced diets use of fish meal from whole fish caught in sustainable fisheries, and not commonly used for human consumption, should be prioritized as well as utilization of trimmings from these fisheries and trimmings from conventional aquaculture. However, concern was raised about trimmings not being a well-defined product showing great variations in composition and quality (amino acids and phosphorous). Focus should also be on improving the diversity of the raw material basket, i.e. increase the adequate options of ingredients to better match amino acid profiles of feed for organic aquaculture. There was a need of harmonizing limits of pigmentation of organic fish as well as consideration of the use of fish meal and phospholipids in shrimp diets. The exchange of fish oil high in omega-3 fatty acids by alternative sources should be adjusted in accordance to development of vegetable or other sources producing these healthy omega-3 fatty acids in favor of keeping the good human health issue of eating seafood.

Sourcing of organic juveniles is a crucial issue. Though organic trout ova was already available the request of 100 % organic juveniles from 1st January 2016 was assessed not realistic in particular for marine species like sole, turbot, sea bream and sea bass due to currently no availability of organic live feed for fish larvae. Specific organic rules are needed 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 incl. phyto- and zooplankton and larval rearing systems. An option might be to start organic rules from fry stage weaned to dry feed. Due to limited possibilities for composition/limited availability of organic feed ingredients concern was expressed about the quality of dry feed for fry in terms of providing essential nutrients.

Recirculation Aquaculture Systems (RAS) and environmental interactions are closely related. RAS produces with minimal environmental impact; i.e. low water usage, prevention of escapes and ingress of pathogens, biosecurity, recycling of water and collection of waste (P is globally limited) - possibly valorized and similar energy use in most situations versus flow through. The main reason for RAS systems only being acceptable for organic juvenile production seems to be more based on consumer perceptions of RAS as a “high-tech-non-natural” system than on scientific information. The consumer survey (WP 3) showed that for most consumers, the production systems are not included in their definition/perception of organic aquaculture. This is probably due to the lack of knowledge about aquaculture production in general. From producer’s point of view, the hatchery should be disconnected from the on-growing phase as for several species it is not economically realistic to produce juveniles in open systems.

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Though not the main factor of **fish welfare**, **stocking density** should be considered in combination with other parameters of water quality, environmental conditions and husbandry practices, and possibly behavior of the fish in the wild. Data on optimal stocking densities are conflicting, though farmers need simple parameters, such as stocking density, to apply. However, more studies are needed about the covariation between fish density on one hand and water quality and a multitude of operational behavioural, physiological and morphological welfare indicators on the other.

Fish welfare is related to a range of parameters, e.g. stocking density, nutritious feed, substrates, light regimes, and being species specific, including conditions during **transportation**. The regulation should differentiate between groups of species, as they can be produced in different ways. As for stocking density metrics there is need of measurable welfare parameters/indicators.

In relation to **health and veterinary treatments** there seems to be a conflict between the current and future regulation of VMPs (all kind of Veterinary Medicine Products) and the organic regulation. The substances of preference in EU Reg. 710/2009 art. 25t a/b/c should be considered as feed raw materials or additives. Further, due to a limited market it is suggested that there is a need for more adequate procedure of authorization of relevant substances for aquatic animals according to the new regulation of VMPs. It was also stressed that anesthetic treatment should not be included in the number of restricted allopathic treatments.

Escapees should be prevented. Species-specific distinctions should be made between escapes of fish and escapes of viable gametes. Escapees might be prevented by robust netting materials to resist tearing or biting by fish and curtain-like egg collectors might be used to mitigate against egg escapee in cages with potential spawners (Atlantic cod and gilthead seabream).

At **slaughter** the most humane stunning methods are assessed to be percussive and electric stunning followed by killing with gill cut. However, alternative stunning methods exist, but await further investigations.

Though the organic principles encourage use of renewable **energy** the regulations give no rules for release of **CO₂** (Carbon footprint) and global warming potential (GWP). Obviously there are insufficient identified criteria and reference points to characterise an environmental friendly food production in relation to climate aspects. Further, there are limitations of the Life Cycle Analysis (LCA) methodology due to lack of clarification on how the environmental impacts should be allocated between co-products in productions and multiple outputs.

In line with the overall organic principles actions should be taken to **recycle waste** of the production. However, a gap of knowledge and technology for recycling of nutrients from aquaculture exists and hence investigations of solutions for collection, de-watering and re-use of waste from aquaculture production are needed.

Off-shore activities are closely related to environmental impact on the **Sea bottom** and the water body. However, limited information on the environmental impact and interactions in relation to cage farming and the sea bottom is available. Further there is a need of

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investigations of the ecological impact of cage farming and foraging devices attracting wild fish.

The **consumers' perception** of what is ECO, organic, fair-trade and sustainable is vague due to lack of knowledge and possibly exposure. Consumers are confused about what organic seafood is, therefore an efficient communication strategy is needed. The image of the EU leaf logo is low in terms of awareness and use because the label is still new and not marketed heavily yet. There are also a large number of organic, environmental/sustainable labels in the market, causing confusion among the consumers. There is much higher awareness for the national labels. Further, the EU label implies the lowest requirements for organic certification in EU. Therefore, it is by nature the least powerful in terms of organic farming practices. However, cultural effects should also be taken into account when considering the organic logo as national labels carry an image of local control, which may be important for developing the organic aquaculture sector. However, transparency, proactive communication and the provision of key information that make sense to consumers may improve the efficiency of the EU leaf logo and the purchase of organic aquaculture products.

A too complex and fragmented management regime seems to be the most important issue of the **institutional framework**, which is aiming at harmonizing the production rules for organic aquaculture in EU. But the uncertainty of the rules and exception deadlines creates a lack of trust and investments. Further the fact that the rules to a very low extent are based on scientific and practical knowledge and experience create constraints for the future development and expansion of the industry. Support policies are particularly needed in this phase of the development for organic aquaculture industry to reach 'critical masses.

A visible and focused information strategy to get consumers familiar with aquaculture (conventional versus organic) is urgently needed. Hence, it should be clearly communicated what is organic aquaculture. The revision of the regulation should provide more homogeneous and species specific rules based on scientific and practical knowledge and experience.

The **ethical** analysis and evaluation revealed a range of potential conflicting interests and needs related to the current framework for organic aquaculture.

The classical dilemma in organic standard setting is visible also in Organic Aquaculture; i.e. increase differences to conventional by stricter standards, taking the risk of losing farmers/producers. Or keep differences at a lower level, causing organic farming closer to conventional, in order to keep, or increase, the number of certified producers. However, this will be at the risk of losing consumers who dislike the 'weak' standards. The critical point is to identify the break even with regard to the levels of the three parameters: 1) Standards, 2) Engaged producers and 3) Consumer trust.

A main aim for the revision is to strengthen and harmonize the rules of organic production and to raise confidence of the consumers to organic production.

However, EU covers an extensive geographic area, which might impose climatic related challenges for small scale organic production systems in rural areas to fulfil the organic rules. 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.

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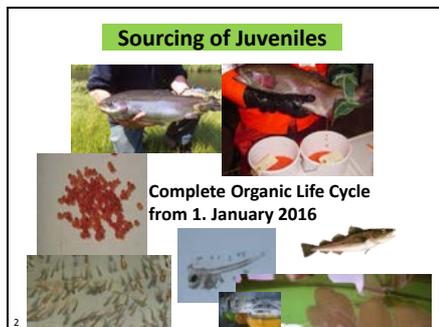
The reviewing of scientific data so far and the feed-back from the 1st stakeholder event clearly revealed a hampering effect of the lack of research and knowledge in organic aquaculture. As seen in other organic sectors, e.g. agriculture a significant development of the European organic aquaculture sector will to a great extent rely on supporting research in key issues challenging the development of organic aquaculture production.

The Power Point presentation of the synthesis of the scientific review process given at the 1st stakeholder event 11th 12th October 2014 in Istanbul: "Production and Socio-economic Issues related to Organic Aquaculture".

Dias 1



Dias 2



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Dias 3

Sourcing of juveniles

Max. non-organic juveniles:

- 80 % by 31.12.2011
- 50 % by 31.12.2013*
- 0 % by 31.12.2015

* Postponed to 01.01.2015 by EU Regulation 1364/2013 of 17.12.2013







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Dias 4

Challenges of Sourcing of Organic Juveniles

1. Inadequate supply of organic juveniles (+ organic trout ova, DK)
2. Lack of specific rules for organic hatcheries (FW & SW) to distinguish organic and non-organic hatcheries, e.g.
 - Breeding (Tools/objectives, selection, robustness etc.)
 - Stocking densities
 - Management
 - Phytoplankton and zooplankton production
 - Essential nutrients
 - "Organic" weaning diets etc. (Hatching → weaning of juveniles)







4

Dias 5

Feed and Nutrition - Carnivorous aquaculture

Sourcing priority of feed ingredients:

1. Organic feed products of aquaculture origin
2. FM & FO from org. aquaculture trimmings
3. FM & FO derived from trimmings of fish caught in sust. fisheries
4. Org. feed mat. of plant origin (max. 60 %)




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Dias 6

Feed and Nutrition

Organic feeding regimes priority:

- Animal Health
- High product quality/human health
- Low environmental impact




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Dias 7

Feed and Nutrition

Sourcing priority of feed ingredients:

1. Organic feed products of aquaculture origin
2. FM & FO from org. aquaculture trimmings
 - Prohibited to feed fish with ingredients derived from the same species
 - Limited organic production → Limited trimmings
 - Below the critical level needed for sustainable manufacturing processes
3. FM & FO derived from trimmings of fish caught in sust. fisheries

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Dias 8

Feed and Nutrition

Fish Meal & Fish Oil derived from trimmings of fish

Considerations:

- Optimum nutrient balanced diet (Amino acids (AA) – Fatty acids (FA)) is crucial for optimum performance
- Fish meal and Fish oil - well balanced nutrient source
- FM from trimmings is lower in protein/essential AA
- Supplementation with AA is prohibited
- FM from trimmings is higher in Phosphorus (P)
 - Decreased performance (growth, health, quality)!
 - Increased environmental impact!

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Dias 9

**Feed and Nutrition –
Alternative options**

FM & FO are limited resources

- FM from whole fish caught in sustainable fisheries may be prioritized
- FM & FO from trimmings for limited use
- Alternative sources of proteins and lipids urgently needed to optimize dietary AA-profile (micro-/macro organisms high in essential AA and FA, plants, PAP etc.)
- Supplementation with essential AA and FA and other essential nutrients derived from processes in line with organic principles



Dias 10

**Feed and Nutrition –
Omnivorous/Polyculture/»Extensive»
Aquaculture**

- Carps, shrimps, tilapia: Natural feed/add. comp. feed
- Molluscs: Extract nutrients from natural local feed web/ organic fish production/water quality issues
- Sea weed: Extract nutrients from the environmental water body/organic fish production







Dias 11

**Health – Veterinary
treatments**

Order of preference:

1. Substances from plants, animals or minerals in a homeopathic dilution (stimulate self-cure)
2. Plants and their extracts not having anaesthetic effects
3. Trace elements, metals, natural immunostimulants or authorised probiotics
4. Allopathic treatments




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Dias 12

Health – Veterinary treatments

Allopathic treatments:

- Max. 2 treatments/year – life cycle > 1 year
- Max. 1 treatment – life cycle < 1 year

Anaesthesia prior to vaccination – counts for treatment?

Parasite treatments

- Max. 2 treatments/year
- Max. 1 treatment – life cycle < 1,5 year

Prolonged withdrawal period for all treatments

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Dias 13

Health – Cleaning and disinfection

Parasite treatments:

- Only Limestone and Dolomite permitted but without anti-parasitic effect

— Need of effective sanitizers for proper management of disease risks in organic open systems, welfare and environmental protection

Substances for consideration in line with organic principles, e.g.:

- Hydrogen peroxide
- Sodium percarbonate
- Peracetic acid and peroctanoic acid
- Calcium hydroxide



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Dias 14

Aeration/Oxygenation



- Only mechanical aerators
- Prefer renewable energy sources
- Pure oxygen only permitted in critical situations

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Dias 15

Stocking density

Salmonids in freshwater (FW):

- Salmon, arctic charr: Max. 20 kg/m³
- Sea- and rainbow trout: Max. 25 kg/m³

Salmonids in seawater (SW):

- Salmon, sea- and rainbow trout: Max. 10 kg/m³

Cod, bass, bream, turbot (SW):

- Turbot: Max. 25 kg/m²
- Others: Max. 15 kg/m²

Carp family and associated Species in polyculture (perch, pike, catfish, coregonids):

- Max. 1.500 kg/ha/y

Consider: Holistic approach



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Dias 16

Welfare

Interactions:

- Feed quality
- Stocking density
- Water quality
- Rearing conditions
- Daylength - Geography
- Physical injuries
- Transportation
- Slaughter methods (preventing suffering in fish, preserving the flesh quality, human safe)



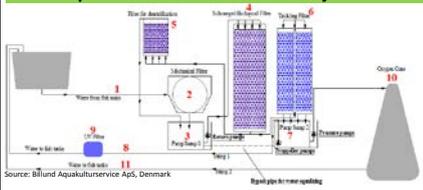
Consider: Holistic approach



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Dias 17

Closed Recirculation Aquaculture Systems (RAS)
NOT permitted – excl. hatcheries & juveniles



Source: Bifund Aquakulturservice ApS, Denmark

RAS: Advantages and Disadvantages. Intensive & energy issues
Consider: Reuse of water – save water resources – renewable energy in line with organic principles.

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Dias 18

Environmental interactions

Escapes
Recycling and waste



18 

Dias 19

Marketing & Sale

- Production vol.
- Distance



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Dias 20

Consumer Perceptions of Organic Seafood and the production systems

1. *Positive to aquaculture* → *Positive to organic aquaculture*
If *Negative* → *Remain Negative*
2. *Positive about organic production* → *Also willing to pay for organic*
- Link stronger at high *Education*, high *Income*, high *Knowledge* about organic and have young *Children*
3. *High Knowledge about organic* → *rational to organic prod.*
Low Knowledge about organic → *Emotional to organic*
4. *Health benefits of organic fish, Naturalness of Local/ Domestic production and Food Safety*



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Dias 21

Consumer Perceptions of Organic Seafood and the production systems

- 5. Only a small segment concerned about *welfare* regimes among consumers in general
- Priority to *Quality, Freshness, Taste*
- 6. Realising aquaculture *protecting wild stocks* → perceive aquaculture *protecting the environment*
- 7. *Missing common understanding* of organic aquaculture; i.e. *Missing distinction* between labels: Organic, Ecological, Green, Sustainable, Fair Trade → *Transparency – Tangibility*





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Dias 22

Consumer Perceptions of Organic Seafood – A Survey

- Low familiarity with labels – in particular the EU leaf
- More familiar with national labels












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Dias 23

Consumer Perceptions of Organic Seafood – A Survey

High priority:

1. No use of *toxic chemicals*
2. *Natural* living conditions
3. Water quality
4. No medicines






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Dias 24

Consumer Perceptions of Organic Seafood – A Survey

Lower priority:

1. Environment
2. Welfare
3. Organic feed
4. Sea cage or pond farming
5. Min. water use
6. Feed utilization
7. Escapees



Lesson:
Consumers' perception of organic seafood seems *not in line* with the EU regulation definition of organic seafood




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Dias 25

Consumer Perceptions of Organic Seafood – Knowledge gaps

1. **Tangible** information about specific production systems and feed → balancing food choices between *moral* and *physical* attributes of organic fish
2. **TRANSPARENCY**: Information transfer and product *labelling* → Organic aquaculture make a difference in the European and global markets

Transparent Information Strategy on Organic Seafood Production




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Dias 26

Economics - and Competitive Position of Organic Aquaculture Products in EU

1. Preliminary main findings of Organic versus Conventional production
1. Higher Production Costs
 - Salmon: 20 – 30 %
 - Trout: 25 – 40 %
 - Sea bass/Sea bream: 20 – 30 %
 - Carp: 10 – 20 %
2. Higher estimated selling price
 - 15 – 30 %






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Dias 27

Economics – and Competitive Position of Organic Aquaculture Products in EU

II. Main reasons for higher production costs of Organic production

1. Lower production intensity → Higher costs/kg prod.
2. Feed price 25 – 30 % higher
3. Higher price of organically raised fingerlings/juveniles
4. Rel. more labour hours and skills – special care/quality/risks



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Dias 28

Institutional Frameworks – Constraints to the Growth of Organic Aquaculture

I. Preliminary identified main constraints of the organic aquaculture regime in Europe:

- Complex and fragmented → Challenging the whole chain
- Bureaucratic production rules and control provisions
- Complexity of bureaucracy hamper the transition to organic certified production
- Lack of national policy support for achieving a critical mass of organic aquaculture production
- Lack of relevant statistics and updated information on organic aquaculture
- Great variation between the countries with respect to standards and certifications hampers export to international markets

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Dias 29

Institutional Frameworks – Constraints to the Growth of Organic Aquaculture

- Cost of certification and requested control programs are relatively higher for small-scale aquaculture producers
- Lack of knowledge/confusion among consumers about organic/conventional and other labels
- Organic aquaculture production may be challenged by stricter regulation for conventional production, which may wipe out some of the differences between organic and non-organic production




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Dias 30

Reflections (I)

- Basically organic production aims natural processes and sustaining the cycle in Nature
- Extensive production in line with organic principles – cf. omnivorous fish, seaweed, molluscs – minor/no input of feed/polyculture
- Contradicting to production of carnivorous fish, i.e. salmonids, bass, bream
 - Pressure on FM & FO
 - Trimmings (P, environment, energy)
 - Transport of ingredients (Carbon-footprint)






Dias 31

Reflections (II)

- Flow through systems for on-growing (No RAS)
 - Risk of infections – limited treatment options
 - Interaction with predators
 - Escapees
- Max. stocking densities: "Extensive"
 - Question mark economical sustainability
- Establishment of robust brood stocks; i.e. stress resilient, disease resistant, ethical welfare
- Critical mass of organic aquaculture production (Ova, juveniles, feed)
- Need of organic aquaculture statistics (database)






Dias 32

Reflections (III)

- Small producers face market barriers
 - Relatively high costs of control and certification
 - Exclude the organic spirit of development rural areas, improve employment and social structures
- Europe has big potential for organic aquaculture products
 - However great imports at competing prices, high carbon-footprint and contradicting organic principles/Institutional frameworks






Dias 33



3.0 Feed-back from 1st Stakeholder Event – Istanbul

The 1st stakeholder event was held in Istanbul 11th – 12th October 2014 as a pre-conference to the 18th IFOAM Organic World Congress.

The stakeholders were presented for the preliminary results of the analyses and integration (WP 4) of the reviews and assessments from WP 2 and WP 3. Based on this information dialogues were facilitated with stakeholders through round table discussions and dialogues in café format, exchanging views and ideas, identifying challenges and suggestions to improve the regulatory framework.

The following feed-back was achieved from the stakeholders.

3.1 Sourcing of organic juveniles

Differences between conventional and organic juveniles

- Organic juveniles deviate from conventional juveniles in their origin from organic broodstock; i.e. no hormone treatments of broodstock, no polyploidy/All female, extra cost (higher price) and higher risk in organic juveniles
- Low availability of organic broodfish and juveniles; particularly for new species not yet in the seed market
- Need for differences in regulations/standards (annexes of the regulations) for different species. The current rules for new species are not realistic and difficult to implement in practice, in particular for marine species like sole, turbot, sea bream and sea bass due to currently no availability of organic live feed for larvae and difficulties with separation of organic and conventional units in RAS hatcheries.
- A separate breeding program for organic juveniles with genetic selection would require starting with new broodstock. Extra cost of an extra breeding program or managing 2 populations for a fish breeding company or hatchery might be difficult to make profitable or sustainable at the current scale of organic aquaculture of most species. If it was possible to use broodfish selected from conventional breeding programs it might prevent losing genetic selection response obtained there. Hence, it could for instance be based on broodfish selected for disease resistance in conventional breeding programs. These may be reared according to organic standards for reproduction and juvenile production.
- The use of antibiotics or other allopathic products for larvae and fingerlings are relatively high in marine species such as seabass and bream. In the opinion of one group member, the current regulations for veterinary treatments are good for on-growing but not for hatchery. For example, “with mixobacteria in trout, you must treat the juveniles with antibiotics two times and there are no problems for the rest of the life”
- In Europe there are 10 hatcheries for sea bream and sea bass, all of them use recirculation systems, where you cannot separate organic and non-organic
- Organic is not always ecologically sustainable; e.g. European eels where juveniles are wild and this is a protected species
- Triploids cannot be organic but triploid fish may be good for the environment in some aspects, because it prevents the reproduction of escaped fish
- Organic feed, in particular for juveniles, may have poorer quality, due to limited possibilities for composition/limited availability of organic feed ingredients. Hence, nutritional value may be lower and following lower production results.

Quality aspects of market sized fish produced from organic juveniles and from conventional juveniles, respectively

- Most did not think that a market sized fish produced from organic fry will have a different quality from a fish produced from conventional fry – and being reared under organic farming conditions for at least the latter 2/3 of its production cycle (Art. 25e, 2). Only the final price may be different
- There are no differences from a product quality (filet etc) point of view, but there are ethical differences
- The main difference in quality characteristics involves the production site, form and system; e.g. diseases and water quality are different depending of the site and the farming system
- Selecting broodstock from breeding program with Genetic selection for resistance to diseases, these may produce more robust juveniles compared to conventional juveniles resulting from breeding with main emphasis on growth.
- A statement from one group member: "I have the impression that what we are doing in this round table is that we are taking conventional aquaculture principles and moving to organics"
- Chemical treatments and feed must be the main differences and must start from nursery, i.e. not from the hatchery.

Any concerns that juveniles shall be organic from 1st January 2016?

- Most stakeholders do not think this to be realistic
- However, species specific; i.e. big problems for marine sp. (bass/bream), but perhaps not for salmon, trout - and carp
- It was mentioned that import of organic trout juveniles from Denmark might be difficult due to body shape, feed conversion efficiency, disease resistance!

Boosting of supply of organic juveniles?

- The **market** "forces" will resolve everything; i.e. *"The expansion of the market could give a boost"*
- Change the rules for marine fish; i.e.
 - 1) Adaptation of the rules to hatchery conditions (sole, turbot, seabass, sea bream,...)
 - 2) No request of separation of organic and conventional in RAS
 - 3) No difference in feeding during live feed feeding phase
 - 4) Differences in feed should only start after finalized live feed phase
- Postpone rules for some species (marine) – and await availability of organic feeds and sufficient scale of production/critical mass and market growth
- Subsidizing (governmental support) of hatcheries and juvenile farmers during transition period until the market become sufficiently big to justify extra costs of organic juvenile production
- Disseminate information about the lack of organic juveniles on the market

3.2 Feed and nutrition

Does it make sense - in line with organic principles - to source feed ingredients for feed for carnivorous fish from the following sources: 1) Organic feed products of aquaculture origin? 2) FM & FO from organic aquaculture trimmings? 3) FM & FO

derived from whole fish and/or trimmings of fish caught in sustainable fisheries? – And in consideration of a) Animal Health; b) High product quality/human health and c) Low environmental impact?

- Use of trimmings from conventional fisheries should be extended (after 31.12.2014)
- Trimmings from conventional aquaculture should still be allowed after 31.12.2014. However, limitations due to, that it is not allowed to use FM from trimmings from a farmed sp. for feed for the same sp.
- Using trimmings was discussed intensively – pro and cons. Contradicting views on amino acid profiles. Trimmings are not a well-defined product, great variations in composition and quality. Obviously, max. limits on phosphorus content in feed is only a problem in Denmark?
- Allow 5 % non-organic compounds to critical life stages
- FM from whole fish from sustainable sources should be used as supplement
- What is the classification for sustainable fisheries? Most for human consumption is classified. MSC should go on the fish species not on the fish meal producer. However, do the consumers distinguish ASC and organic?
- Crucial to improve the diversity of the raw material basket, i.e. increase the potential adequate ingredients to better match amino acid profiles of feed for organic aquaculture
- No limits of types of raw materials, i.e. PAP, blood products, microalgae, insect meal (however, in-appropriate FA profile), processed vegetable protein (soy protein concentrate). Supplement from fermentation e.g. Histidine (but also other amino acids) should be allowed
- Jack mackerel could give the histidine, but this currently only goes to human consumption. However, histidine from FM from South America cannot be used because of the current fishery stop and in general because of using ethoxyquin (preservation)
- Due to limitations in sourcing of ingredients for feed for organic carnivorous fish: Should carnivorous species be in organic aquaculture at all? The new regulation will kill carnivorous aquaculture?
- Need of harmonizing limits of pigmentation of organic fish, i.e. max. amount of ppm astaxanthin in feed. Some national regulations allow 100 ppm, while e.g. Danish authorities have interpreted the EU regulation as max. 20 ppm!
- Need of removing barriers (crosscutting regulations) regarding use of different feed materials (plant), insects, worms, mussels in organic feed
- Need for a lower limit value for ethoxyquin, due to analytical uncertainties/deriving from an ingredient. For GMO the limit is 0.9%.

3.3 Health – Veterinary treatments

Will it be realistic/sustainable to farm organic fish without any medical treatments – and will there be a future for herbal medicine in organic aquaculture?

- Anesthetic treatment shall not be included in allopathic treatment limitation
- There is a conflict between the VMPs (all kind of Veterinary Medicine Products) current and planned future VMP regulation and the organic regulation:
 - 1) The substances of preference (article 25t a-b-c) should be considered as feed raw material or additives.

2) Due to a limited market aquatic animals should have a more adequate/easier procedure of authorization of relevant substances according to the new regulation on VMPs

- Reconsider the setting of with-drawal period according to the VMP regulation, i.e. if a with-drawel period is not defined for a species or a product you can multiply by 1.5 the with-drawel period for a similar product registered for another species
- Herbal medicine may play a significant role as immunostimulant and as treatment tool in future organic aquaculture.

3.4 Stocking density

How do you perceive stocking density in relation to fish health and welfare/wellbeing - implications for growth rate, behavior, aggression, metabolic capacity?

- Stocking density is not the main factor for fish welfare; but should be considered in combination with other parameters of water quality, environmental conditions and husbandry practices. Although farmers need simple parameters to apply
- Establishment of a database with specific information among species and rearing systems in order to set reliable parameters of stocking density for practical use
- Views were put forward that stocking density of organic and conventional aquaculture should be distinguished as it is in other organic productions. It should be completed by other indicators of water quality (e.g. water renewal, oxygen content, nitrogen compounds) and fish condition and management practices
- Contradicting wiews were put forward that there should be no differences in stocking density limits between organic and conventional aquaculture. Control of fish welfare (presence of injuries, diseases occurrence, survival) and veterinary treatments are more important. Stocking density is less important than survival rate, growth and feed conversion rate, which are indicators of fish welfare
- Use of space and water is also very important and should be considered a limiting factor. A specific stocking density limit in itself is not sufficient, but the behavior of the fish in the wild should also be accounted for.

3.5 Welfare

Welfare of organic versus conventional produced fish?

- Different from species to species (space, wellbalanced feed)
- New production segment with further need to prevent disease – economy is important
- Max. number of treatments might give welfare problems; e.g. ineffective treatments (homopatic) – and keeping organic certificate
- Better trained staff in organic (know about careful handling etc.)
- Need of measurable welfare parameters/indicators
- Welfare important for the consumer (emotional)
- Think of fish as humans,- respect of animals
- We shall not mimic nature, because it might not be the best for the fish
- Accept eyestock ablation if there is no other possibility
- Sea lice problem can be solved with laser, - there is a continuous development of new technical solutions

Relations between welfare and the needs of fish, such as stocking density?

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- Transport and harvest of fish has impact on the quality
- Perception is different in different countries
- Needs more study
- Optimal feed (Histidine) is crucial
- Regulation should cover group of species, as they can be produced in different ways.

3.6 Environmental interactions (incl. Recirculation Aquaculture Systems (RAS))

RAS produces with minimal environmental impact: low water usage, prevention of escapes and ingress of pathogens, recycling of water and collection of waste (P is globally limited) - possibly valorized and similar energy use in most situations versus flow through.

What is your opinion about the regulation related to RAS knowing the pros and cons?

How many and which articles of the European regulation on organic aquaculture should be amended in order to allow RAS for on-growing farming?

- What is “a closed RAS” definition and what is the renewal rate of a ‘closed’ system (not acceptable for organic label) compared to an open one (accepted)?
- Why RAS systems acceptable for fingerling and juvenile production are not acceptable for larger fish production? It seems that it is more a problem of acceptability by the consumer than based on scientific information. From producer’s point of view, hatchery should be disconnected from the on-growing phase because, for several species, producing juveniles in open systems is not realistic (economically)
- RAS have important advantages as biosecurity, water control, protection from escapees, becoming energy efficient
- The main reasons why RAS are not accepted as an ‘organic’ system are the high level of technology (very complicated system with a lot of tubes and treatment systems...), which makes it looking like a non “natural” system. The image of ‘natural’ is very important in the mind of the consumers. Some consumers think organic systems have to be “un”controlled. The rearing environment (water quality) of the fish (O₂, CO₂, TAN... concentrations) is the most important concern regarding its welfare condition, as well as the biosecurity aspects (no diseases). Other items, which are not directly linked to the fish welfare as the environmental impact (footprint, land use, water use, escapees) of the production and the quality of the product (flesh quality) should be included in the certification criteria
- What is animal welfare and what are the scientifically documented available criterias? There is lack of knowledge and of communication on the indicators
- The consumer acceptance of the label is a key question, but the average scientific awareness of the consumers concerning the above questions is very limited. Therefore the information – education process through the popularisation and dissemination of the knowledge is a key aspect to consider. It seems that even general knowledge on aquaculture... is missing
- Why is it such a huge discrepancy between the production methods accepted for vegetal production compared to animal production and more specifically aquaculture productions? Namely, very intensive tomato productions in very intensive conditions in greenhouses are accepted for an organic label, but not RAS; pig or other animal castration is accepted but not shrimp eyestalk ablation...

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- In the current regulation for organic products, the rules are absolutely not homogeneous with some very detailed information besides very general concerns.

In the 3 groups participating in the round table, more or less 50% pros and 50% cons RAS as a tool to produce organic fish, which is consistent with the IFOAM survey that was carried out some months ago.

3.7 Consumer perceptions and economics

The EU Logo and the requirements it represents regarding production methods are different to the requirements needed to receive private and national labels. How do you think this may influence the perception of labels by consumers and retailers when choosing which organic seafood products they buy?

Main feed-back regarding the EU leaf logo:

- The image of the EU leaf logo is low in terms of awareness and use because the label is too new. Time will lead to exposure, awareness, familiarity, trust and use of the label
- Co-branding the EU leaf logo with national labels that certify organic products will assist in increasing awareness and trust
- The EU label implies the lowest requirements for organic certification in EU. Therefore, it is by nature the least powerful in terms of organic farming practices
- Retailers and HoReCa (Hotels, Restaurants, Cafes) are very important in the process of promoting the EU logo, because they do not only focus on specific products that carry the logo, but also on total self-branding as a carrier of organic products. They have much impact on consumers and they are the gate-keepers for products to move from production to the consumers
- A cultural effect should be taken into account when considering the EU logo too. National labels carry an image of local control as well, which is important in EU countries. However, the EU logo does not carry a specific sense of origin for the products that carry it. This issue is related to consumer perception and may not be realistic in terms of actual production origin. However, if it is perceived by the consumers, it is relevant for a profitable organic aquaculture sector
- Transparency, proactive communication and the provision of key information that make sense to consumers may improve the efficiency of the EU leaf logo.

Main feed-back regarding the consumers' impression that only wild fish can be organic:

- Consumers have a vague perception of what is ECO, organic, biological, fair-trade and sustainable, due to lack of knowledge and linguistic confusion among languages in EU. Therefore, if ECO was consistently used for fisheries and organic for aquaculture, consumers would have less difficulties in gaining a direct understanding of what is organic and what is not
- Consumers are confused about what is organic, mostly due to information provided from agriculture. The two productions differ significantly and few organic agriculture products are identical in conventional and organic. Therefore, communication about organic food in general should be better coordinated to differentiate between agriculture and aquaculture
- Some fish species have a natural life that is significantly far from the one they have while being produced in a static fish farm. So, the associations that informed consumers

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may make between organic and natural (and elements included in the perception of naturalness) may be too weak, once they realize that the standard organic principles are far from what organic production is covering

- Organic aquaculture faces challenges to reach the demands based on the main organic principles. Therefore, organic certified aquaculture products are 'less organic' than agriculture products. This may lead to the confusion regarding organic seafood and could put the image of organic food production in general at some risk of losing the strong connection to the main organic principles.

3.8 Institutional frameworks

The current EU regulation is aiming at harmonizing the production rules for organic aquaculture in EU. The political strategies have anticipated (for long time) significant increases in organic production. Why has the production not increased? Might it be due to:

- **A too complex and fragmented management regime?**
- **Too bureaucratic production rules and control provisions?**
- **Lack of national policy support for achieving a critical mass of organic aquaculture production?**
- **That the regulation is too costly to meet?**
 - Uncertainty of the rules and on exception deadlines, which creates a lack of trust and investments
 - Rules are too ambitious, i.e. the rules have been developed too much and too detailed before sufficient scientific and practical knowledge is available
 - Too general for too many species; i.e. rules are based on knowledge on salmonids and extended to other species, which may have other requirements
 - Lack of specific rules for hatcheries
 - Need of visible and focused information strategy to get consumers familiar with aquaculture (conventional versus organic)
 - Need of support from the government for conversion to organic
 - Lack of profitability and high risk for producers (a lot of certified fish is still sold as conventional)
 - Organic aquaculture in competition with conventional and with wild fish

Suggestions for improvements:

- Specific rules for hatcheries and juveniles
- More technical species-specific rules
- Allow parallel production (member state issue)
- Support policies for organic aquaculture to reach 'critical mass' (conversion, maintenance investments; certification costs, promotion and marketing)
- More information and education for the consumers
- The revision of the rules should define and communicate what is organic aquaculture

4.0 Conclusion: Recommendations - Research gaps

Based on the analysis of the preliminary information provided by WP 2 and WP 3 and feedback from the 1st Stakeholder event in Istanbul 11th -12th October 2014 the following issues should be considered to underpin future growth of the European aquaculture sector.

Nutrition

- 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 and not commonly used for human consumption, 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 shrimp diets 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 as ingredients in feed for organic aquaculture
- 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 when considering ingredients in feed for organic aquaculture
- 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)
- Prioritize research in alternative sources of omega-3 fatty acids (HUFAs)
- Chemically well-defined analogic substances of minerals and vitamins may be considered as ingredients in feeds for organic aquaculture if the natural substances are unavailable.

Organic juveniles

- Except for already available organic trout ova it seems difficult to fulfil the request of 100 % organic juveniles from 1st January 2016 in particular for marine species like sole,

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turbot, sea bream and sea bass due to currently no availability of organic live feed for larvae

- Specific organic rules are needed for managing the life cycle stage between the hatching and the weaning of juveniles for specific species in fresh water, but particularly marine species
- The current regulation does not distinguish between organic and non-organic hatcheries incl. phyto- and zooplankton and larval rearing systems
- An option might be to start organic rules from fry stage weaned to dry feed
- Due to limited possibilities for composition/limited availability of organic feed ingredients concern is raised about the quality of fry dry feed in terms of providing essential nutrients
- If available, domesticated and unrelated broodstock, preferably selected for relevant robust traits (survival, disease resistance and growth) should be used in breeding for organic seed
- Need of defining breeding objectives and implement cost effective breeding strategies that control inbreeding rate at a sufficient low level (<0.5% per generation) to secure adequate genetic material specifically for organic aquaculture.

Recirculation Aquaculture Systems (RAS) – Environmental interactions

- RAS produces with minimal environmental impact: low water usage, prevention of escapes and ingress of pathogens, biosecurity, recycling of water and collection of waste (P is globally limited)
- Similar energy use in RAS in most situations versus flow through systems
- The main reason for RAS systems only being acceptable for organic juvenile production seems to be more based on consumer perceptions of RAS as a “high-tech-non-natural” system than on scientific information
- From producer’s point of view, the hatchery should be disconnected from the on-growing phase as for several species it is not economically realistic to produce juveniles in open systems
- Need for more knowledge on fish welfare in RAS
- Further knowledge is needed about RAS and IMTA and the potential use of these concepts in organic aquaculture.

Welfare

- Data on optimal stocking densities are conflicting. More studies are needed about the covariation between stocking density on the one hand and water quality and a multitude of operational behavioural, physiological and morphological welfare indicators on the other
- The potential benefits of providing fish with access to nature-like substrates are species specific. More data are needed on type of substrates for specific species. Current knowledge suggests e.g. salmonids and maybe other species (e.g. wrasse) may not have a preference for substrate *per se*, but a preference for shelter, that could be overhead, floating or benthic
- More knowledge is needed on the significance of light regimes requirements on the welfare and performance in organic aquaculture.

Health – Veterinary treatments - Biosecurity

- Anesthetic treatment should not be included in allopathic treatment limitation
- There is a conflict between the current and future regulation on VMPs (all kind of Veterinary Medicine Products) and the organic regulation:
 - 1) The substances of preference (article 25t a-b-c) should be considered as feed raw material or additives
 - 2) A more adequate procedure of authorization of relevant substances according to the new VMP regulation might be considered in relation to organic aquaculture
- Reconsider the setting of with-drawal period according to the VMP regulation, i.e. if a with-drawel period is not defined for a species or a product you can multiply by 1.5 the with-drawel period for a similar product registered for another species
- Herbal medicine should be further investigated as it may play a significant role as immunostimulant and as treatment tool in future organic aquaculture.

Transport

- Excessive changes in water temperature and pH during transportation must be avoided
- Smolt densities of up to 70 kg/m³ by road transport for up to 90 minutes did not compromise fish welfare
- Open-hold wellboat transport, densities of up to 150 kg/m³ for more than 10 hours had no significant effect upon salmon welfare
- Max. density with transportation of fry might be set to 10 kg/m³
- The loading phase appears to be more detrimental to welfare than the transport phase and well boat transports seemed to have an important recovery function
- The effects of isoeugenol on large scale transport of smolts need further investigation
- The potential welfare costs/benefits of large scale live chilling during transport need to be investigated in greater detail for adult fish.

Killing – Slaughter

- When properly done the most humane stunning methods is percussive and electric stunning. The methods should be followed by killing with gill cut.
- Throughout storage prior to slaughter water quality should be monitored and continuously adjusted accordingly
- Use adequate pump equipment with care and only trained staff should manage such equipment
- Personnel in slaughtering should be regularly (annually) trained regarding fish welfare and equipment
- More investigations are needed to evaluate alternative stunning methods regarding humane slaughter (e.g. CO₂, alternative anaesthetics)
- The use of electric stunning is considered as humane, but today the method is complicated and neither user friendly nor easily applied commercially
- Alternatives to waiting cages should be investigated

Escapee

- Species-specific distinctions might be made between escapes of fish and escapes of viable gametes

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- Efforts should be put on prevention of escapees, i.e. putting requirements for the physical design of the installation of net cages, i.e. calculation and design, operating and maintenance requirements
- Specifications should be put on robust netting materials to resist tearing or biting
- Curtain-like egg collectors might be used to mitigate egg escapee in cages with potential spawners (Atlantic cod and gilthead seabream). The commercial efficacy needs to be tested.

Energy consumption – CO₂ – Life Cycle Analysis (LCA)

- Need of defining criteria and reference points for an environmental sustainable food production
- Need of more research on LCA methods to evaluate properly environmental impact and carbon foot print.

Recycling and waste

- Need of more knowledge and technology for recycling of nutrients from aquaculture
- Need of more investigations of solutions for collection, de-watering and re-use of waste from aquaculture production.

Sea bottom

- Environmental impact and interactions in relation to cage farming and the sea bottom needs consideration
- Ecological impact of cage farming and wild fish attracting device needs consideration.

Consumer´s perception

- The consumer´s perception of what is ECO, organic, fair-trade and sustainable is vague due to lack of knowledge and linguistic confusion among languages in EU
- Consumers are confused about what is organic, and hence information about organic food should be significantly focused
- An efficient communication strategy is urgently needed
- A cultural effect should be taken into account as national labels carry an image of local control, which may be important for a developing organic aquaculture sector
- Transparency, proactive communication and the provision of key information that make sense to consumers may improve the efficiency of the EU leaf logo and the purchase of organic aquaculture products. It should be clearly communicated what is organic aquaculture.

Institutional frameworks

- Too complex and fragmented management regime seems to be the most important issue of the institutional frameworks, which is aiming at harmonizing the production rules for organic aquaculture in EU
- Uncertainty of the rules and on exception deadlines creates a lack of trust and investments
- The rules are not based on sufficient scientific and practical knowledge and needs to be differentiated according to different species/groups

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- Support policies are needed in this initial phase for the organic aquaculture sector to reach 'critical mass'.

Ethics

The ethical analysis and evaluation revealed a range of potential conflicting interests and needs related to the current framework for organic aquaculture. The Following dilemmas and issues need further attention and clarification when considering future regulation of organic aquaculture:

- The classical dilemma in organic standard setting is visible also in Organic Aquaculture; i.e. increase differences to conventional by stricter standards, taking the risk of losing farmers/producers, or keep differences at a lower level, not necessarily minimum, but closer to conventional, in order to keep, or increase, the number of certified producers, but at the risk of losing consumers who dislike the 'weak' standards? The critical point is to identify the break even with regard to the levels of the three parameters: 1) Standards, 2) Engaged producers and 3) Consumer trust, which includes:
 - ▶ How to gain consumer trust in organic aquaculture if the differences to conventional systems are low? What to inform consumers about it there are few differences?
 - ▶ How ensure increase in organic aquaculture if large differences to conventional leads to few producers being interested?
 - ▶ On the other hand, how to keep or create an interest among those organic producers who strive for a substantial difference and contribution?
- Fish welfare need to be defined in relation to each species, and welfare indicators are needed
- Stocking density includes several interconnected rearing parameters (water quality), which addresses welfare as well as other ethical issues
- Impact of stocking density on fish welfare is difficult to measure, and opens for a range of ethical considerations
- The definition of 'unnecessary suffering' as related to rearing systems, consumer perceptions and regulations (Organic, EU Slaughter directive as well as Treaty of Lisbon) needs further clarification
- Stunning followed by slaughter can be performed without causing (much) stress and pain, but legislation still allows methods that do (CO₂). This needs to be addressed in the organic regulations
- In particular regarding farming of species fed on animal protein: Is this the best possible use of global resources? Are arguments in favour of feeding cattle soy proteins that humans could eat instead and feeding carnivorous fish fishmeal convincing? If yes, are they so strong that it also justifies the suffering and stress we cause individual animals? Is there a morally/ethic relevant difference between cows and fish? Are fish less worthy of ethical consideration than other animals? If not, what is the alternative? What is the ideal organic system? Cattle eating mainly grass we can't eat, monogastric animals (pigs) mainly eating our waste and fish fed mainly on alternative protein sources? If so, what is the role of organic regulations in promoting such a shift?

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.

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However, 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.

APPENDIX

A1.0 Nutrition

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. (9)).

"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. (15d)).

"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." (EC 834/2007, rec. (16 2(e) (ii)).

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.

A1.1 Carnivorous fish and shrimps

A1.1.1. Introduction: Present regulations

1. "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." (EC 889/2008, art. 25k).

2. If feed mentioned above (1a – 1d) is not available, fishmeal 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.

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

A1.1.2 State of the art

Organic aquaculture is a value based and 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.

A1.1.2.1 Fish Meal Replacement

It is a fact that fishmeal and fish oil 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.

A1.1.2.1.1 Current scientific knowledge

Replacing fish meals 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 and sea bream 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 immunocompetence (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 vegetable sources (corn gluten, wheat gluten extruded pea and rapeseed) without compromising digestive process, but the in-take of fish feed significantly decreased (Santigosa et al., 2011b). A total substitution induced a strong reduction of the protease activity.

High replacement ratios to meet the high protein requirement of fish require that anti-nutrients, such as trypsin inhibitors, tannins, lectines or glucosinolates (Chebbaki et al., 2010) are efficiently removed from alternative plant protein ingredients. The dietary content of indigestible substances should be minimized to improve the efficiency of the feed and to balance with other objectives (e.g. animal welfare, environment) in organic aquaculture.

Extrusion processing could be used to obtain vegetable products with extremely low levels of anti-nutritional factors and to increase the nutritional value of protein-containing ingredients (Chebbaki et al., 2010). 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 Seilliez, 2010; Wilson, 2002). The use of agar coated crystalline-amino acids in sea bream juveniles was 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 salmon and trout 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 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 seabream.

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 dietary 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 also be 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 fishmeal with up to 25–35 % (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).

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The most important shrimp species in aquaculture are *L. vannamei* (White shrimp), *P. monodon* (Giant tiger shrimp).

Although they are all benthivore species, they have different diets in their natural habitats:

- *L. vannamei* is an omnivorous benthivore, and mainly feeds on living preys and detritus (FAO 2011);
- *P. monodon* is a carnivorous benthivore and 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 protein level for giant tiger shrimp is 40% - 50% of meal content (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 can vary for:

- *P. monodon* between 35 and 50% of the dry matter in feed (Fox et al., 1998; Cousin, 1995; FAO 2011; Dayal et al., 2003; McVey, 1993).
- *L. vannamei* between 20 and 30% of the dry matter in feed (Velasco et al. 2000; Cruz-Suarez et al. 2000; Kureshy and Davis 2002).

Experiments of fish meal substitution were also conducted on carnivorous species of penaeid 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 (*Penaeus monodon*) by Paripatananont et al. (2001). Generally, high dietary concentrations of soy bean products in some species of shrimp negatively affect palatability. Anyway, Paripatananont et al. (2001) showed that up to 17.5% inclusion of SPC in shrimp feed does not adversely affect the feed intake and the growth rate, while further progressive levels of substitution lead to impaired 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 environment 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 because 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).

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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 25k(b) fish meal and fish oil from organic aquaculture trimmings is prioritized followed by 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 P-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. 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). There are different chemical forms of phosphorus in the diet. Very 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 P levels, can give P 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

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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 % is processed into fillets, yielding about 50-60 %, leaving about 40–50 % trimmings. The amount of trimmings available for manufacturing of fish meal and fish oil may therefore be about 5–6,000 t. Assuming 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 requires relatively high dietary protein content, i.e. 38-48 % of the diet, depending on fish size, with the highest requirement and quality for fry and brood-stock. Indeed, 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 was 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 and oils, 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 fishmeal 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 salmonid and other carnivorous fish species (such as sea bass and sea bream) feed. 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 (e.g. 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

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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 used to as feed for Zooplankton, which again is fed to fish. The idea is to harvest from the first trophic level or cultivate in a close system and provide feed ingredients for farmed fish by culturing microalgae. Although the benefit of using different micro algae in an organic feed need to be demonstrated scientifically for each aquaculture species, as well as a mass production and an economic model have to be developed. However, the appropriateness of using cultured microalgae in feed for organic aquaculture needs further consideration related to organic principles and the use of renewable sources.

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 European sea bass was demonstrated on immune and digestive fish system. (Cahu et al., 1998). Modern process and algae cultivation in photobioreactor or fermentation system can provide algae in a flour form which can be used similar to fish meal for the production of formulated pellets. The chemical composition of microalgae varies depending on species, cultivation parameters and the potential as a feed ingredient varies accordingly (Skrede et al., 2011). A diet with the microalga T-Iso (*Isochrysis spp.*) resulted in a better growth rate of juveniles of gilthead sea bream than control diets. Further, the chemical composition of sea bream fillets also met the requests of the consumers, although the level of protein was low compared to conventional diet and the level of fats was higher. Diets containing T-Iso was highly digestible, and resulted in the best performance of fish fed on a diet based on 70% of microalgae, probably due to its high protein efficiency (Palmegiano et al., 2009).

Addition of algae in a nutritional assay has been conducted with rainbow trout fry (*Oncorhynchus mykiss*) 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 (*O. mykiss*) 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 (*Sparus aurata*) pellets showed better performances than control diets. The best performances of fish fed on 70% algae diet was probably due to the protein 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 carp diets in Japan and China. 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 and African catfish 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 and blue tilapia 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 (Nandeeshia et al., 2000). Ng et al. 2001 demonstrated that *T. molitor* larvae meal was highly palatable to the African catfish (*Clarias gariepinus*) 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 (*Oncorhynchus mykiss*) 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. Particularly, worms and insect larvae/pupae can be considered as natural fish feed in the wild life. The CAB Abstracts database contains some 700 records describing research on alternative protein sources for use in aquafeeds.

A1.1.2.1.2 Current practices

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 seems impossible for the feed industry to source ingredients strictly according to the regulation already next year while maintaining consideration of fish performance, health and welfare as well as environmental friendly production.

A1.1.2.1.3 Gaps between organic regulation – scientific and industrial standards

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 (P) in the fish meal derived from trimmings might conflict with national environmental legislations, because this may result in too high P-concentrations. As mentioned above, fish meal from trimmings is lower in protein and higher in phosphorus content compared with high quality fish meal. Using this meal for feeding fish puts limitations on the inclusion level of phosphorus 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.

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 fishmeal 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.

As regards penaeid shrimps, considering a semi-intensive farming in ponds, it seems difficult to meet the animal's nutritional requirements considering the limitation of 10% fishmeal 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).

A1.1.2.1.4 Conclusion: Recommendations and research gaps

- Sourcing of feed ingredients for organic aquaculture need to be re-considered and supported by experimental data to secure compliance with organic principles of fish welfare and environmental friendly production.
- At least until more knowledge is available fish meal and fish oil derived from industrial fish caught in sustainable fisheries, and not commonly used for human consumption, 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, and possibly 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 farming?, essential amino acids and lipids obtained by fermentation, worms and insects or other similar procedures close to the organic principles should be considered.
- The issue of timing of amino acids from non-marine protein sources entering the blood should also be further investigated and taken into consideration when evaluating feed ingredients.
- Procedures in compliance with organic rules for removal of anti-nutrients in plant sources should be developed and considered for utilizing more organic crops as feed ingredients.
- 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

A1.1.2.2 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 as well as these plankton are strategic ingredients to be used at critical stages of the life-cycle, when optimum performance is required.

A1.1.2.2.1 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. 3.1.1.

A1.1.2.2.2 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). 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

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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.

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 $\Delta 5$ - and $\Delta 6$ - desaturase activities in salmon is depressed when fed high dietary levels of FO, while vegetable oils increase the capacities (Ruyter et al., 2003, Moya Falcon et al., 2005, Kjær et al., 2008).

Several studies have shown that there are species differences in the capacities for conversion of essential FAs (Sargent et al., 2002). A recent study (Berge et al., manuscript) showed that rainbow trout had apparent retention of 22:6n-3 in the range 129-194%, while the salmon obtained values in the range 86-120%.

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 & 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 biopotency than their n-3 homologues (Lands, 1992).

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.

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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 preferred. 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, 2000b). 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 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, such 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). 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 and 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) (Palmelegiano et al., 2009).

Progressive substitutions of fish oil with Cottonseed Oil (CSO) did 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 with sesame oil (SO), canola oil (CO) and soybean oil (SBO), respectively, 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 soyabean oil (SO), rapeseed oil (RO) and linseed oil (LO), respectively or a mixture (Mix) of them. 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, seabass liver showed much higher lipid contents than seabream, 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 vegetable oils (rapeseed, linseed and olive oil), up to 600 g kg⁻¹ of dietary oil, significantly reduced EPA and DHA and increased 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 were 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 increased 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 synthesize 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 *P. monodon* and *L. vannamei* 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 because 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 as follows:

P. monodon – 1% of the diet for post-larvae (Paibulkichakul et al., 1998) and 1.25% for juveniles (Chen 1993); *L. vannamei* – 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.

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For *P. monodon*, cholesterol need is lower, but it 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 *L. vannamei*, there is a relationship between cholesterol and phospholipids. A diet with no phospholipids required 0.35% cholesterol, whereas a diet with 5% phospholipids required 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 for 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 (*Sparus aurata*) performances and chemical composition of fillets. Best fish performances were observed when fish were fed on 70% algae diet probably due to highest amount of saturated fatty acids, which mainly was due to myristate and palmitate acid (Palmegiano et al., 2009). The use of heterotrophic algae source *Schizochytrium* or *Cryptocodium cohnii* in the early Sea bream stage showed an important potential of these strains 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).

A1.1.2.2.3 Current practices

Cf. A1.1.2.1.2

A1.1.2.2.4 Gaps between organic regulation – scientific knowledge and industrial practises

Conflict between Omega-3 fatty acid (Highly unsaturated fatty acids – HUFAs) content in fish flesh, and related human and fish health and welfare concerns on one hand and the regulation requesting exchange of fish oil with plant oil mostly low in Omega-3 fatty acids due to environmental concerns on the other. The promotion of (organic) fish as healthy may be impeded as the content of favourable fatty acids are reduced. Hence, there is a need of research and development of plant oils high in Omega-3 fatty acids. Furthermore, a conflict with the organic regulation in relation to the use of cholesterol and lecithin as feed additives is possible.

A1.1.2.2.5 Conclusion: Recommendations and research gaps

- 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).
- Prioritize 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 should be considered.

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- For preference, lecithin from organically certified sources, such as organic soybean, may be used as phospholipid source following mechanical extraction. If unavailable, non-organic natural sources may be used provided they are of non-GMO origin

A1.1.2.3 Mineral and vitamin supply

Information on requirements of minerals and vitamins for salmonids is limited. Some knowledge is available for marine finfish such as sea bass and sea bream.

Dietary supply in organic production is preferred from sources of 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))).

There are factors that complicate the assessment of dietary requirement. 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 marine finfish such as sea bass and sea bream. For sea bream Oliva-Teles (2000) reported a dietary phosphorus requirement around 0.75%. Bioavailability 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 *P. vannamei* that in absence of dietary calcium supplementation, the adequate dietary phosphorus amount was 0.34%, although the minimum level of dietary phosphorus for maximum growth of *P. vannamei* was dependent on the calcium content in the diet. Anyway, shrimp diet containing 3% or more of calcium should be avoided. In *P. monodon* Ambasankar et al. (2006) estimated that the best 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.

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Of the water soluble vitamins, the B-complex is 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 strains might 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 marine finfish such as sea bass and sea bream is very scarce. A dietary requirement for vitamin B6, pyridoxine, has been demonstrated in several species of freshwater and marine fish. 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 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 for freshwater fish and 12.6 – 47 mg of AA/kg 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 Cyprinids and some Acipenserids, most finfish cannot synthesize AA. Documented pathological effects of vitamin C (ascorbic acid) 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 requirement for seabream is less than 25 mg/kg. 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 was 5 mg of AA/kg, 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 was sufficient for sea bass.

Vitamins have pivotal roles to ensure good survival rates in aquaculture also in shrimps diets. Indeed, vitamin C deficient diets in *Penaeus vannamei* caused 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) also estimated that the minimum dietary vitamin C levels required for normal survival of *P. vannamei* specimens of 0.1g and 0.5g were 120mg ascorbic acid-equivalent (AAE)/kg and 90 mg AAE/kg, respectively, showing that dietary vitamin C requirement of *P. vannamei* decreased with increased size. Furthermore, there are evidences that dietary ascorbate enhances immune responses in *P. vannamei* (Lee and Shiau, 2002).

Vitamin E, as a fat-soluble compound, is the most effective lipid-soluble antioxidant 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 *P. monodon*, 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, coloring 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 performed by Gensch et al. and published by BASF around 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 *P. monodon* is guaranteed by dietary levels of 50mg kg⁻¹ of astaxanthin (Menasveta et al., 1993). Also survival and growth rates of post-larvae increase according to dietary astaxanthin in *Penaeus monodon* (Merchie et al., 1998) and in *Litopenaeus vannamei* up to supplementation levels of 200 and 400 mg kg⁻¹ (Niu et al., 2009).

A1.1.2.3.1 Current practices

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.

A1.1.2.3.2 Gaps between organic regulation – scientific and industrial standards

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.

A1.1.2.3.3 Conclusion: Recommendations and research gaps

Chemically well-defined analogic substances of minerals and vitamins required to maintain health and welfare should be authorised for use if the organic sources and natural substances are unavailable.

A1.1.2.4 Sustainable fisheries

In order to ensure the sustainability of fisheries, different systems have been introduced at different levels, like 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.

Advices 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, 2011a). The international fishmeal and fish oil organization (IFFO), who represents the fishmeal 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 (<http://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

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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 of 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).

A1.1.2.5 Sustainability

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.

LCA was used to compare three representative carnivorous finfish production systems: rainbow trout (*Oncorhynchus mykiss*) in freshwater raceways in France, sea-bass (*Dicentrarchus labrax*) in sea cages in Greece, and turbot (*Scophthalmus maximus*) in an inland recirculating system close to the seashore in France (Aubin et al., 2008). The results showed 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 contributed to the climate change and acidification impact throughout feed production and net primary production use (NPPU), the turbot recirculating system contribution was accounted to its high energy consumption.

However, the limitations of the LCA methodology should be noticed, 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).

A1.2 Carp

Common carp (*Cyprinus carpio* L.) is a good example of freshwater aquaculture characterized by low labour productivity and low capital intensity, serving mainly local markets (Guillen, Motova 2013). Limited demand and strong international competition is limiting the profitability and growth of production. However the extensive and artisanal production may play a role in environmental and recreational aspects (e.g. regarding biodiversity and preserving cultural landscapes).

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In 2010, the contribution of carp to total EU aquaculture production and its value were 5 (66 thousand tonnes) and 4% (133 million EUR), respectively (Gillen, Motova 2013). Common carps were mostly produced in the Czech Republic (27 %), Poland (23 %), Hungary and Germany (15 % both). However, organic carp production is reported only from Hungary (~ 700 t), Germany (~ 200 t) and Austria (~ 150 t). Pond areas with organic carp production occupy 4,700 and 550 ha in Hungary and Austria, respectively, which represents approximately 20% of the total fishpond area in both countries (Varadi, Phuong 2007).

Carp represents an ideal candidate for organic status since it is low in the food chain, feeds naturally and in pond farming, it has a minimum impact on the environment. Cyprinids (carp family) are by far the largest family of farmed finfish (20.4 million tonnes or 36.8%). These are mostly produced by Asian family enterprises and consumed locally. Typically, they apply many organic production principles often using polycultures or multicultural systems that include rice, ducks, geese or pigs, and give a general priority to fertilising rather than feeding. Nevertheless, these systems would certainly still face several obstacles if they were to seek organic certification, mainly due to gaps in quality management and traceability of different inputs.

In 2001, totally 200 - 400 ton of organic carp and associated freshwater species such as tench (*Tinca tinca*) were produced from Austrian and German farms for sale mainly to domestic markets. Organic farmers and organic farming associations in Austria and Germany first started to develop extensive "organic" carp production systems in the early nineties as a side activity, selling regionally to farm stores and weekend markets. Organic aquaculture certification programs, crops and standards related to carp included Naturland (Germany), ERNTE (Austria), Bioland, Demeter and Biokreis (Germany) (Tacon, Brister 2002). Naturland launched their activities in 1995 with development of organic aquaculture standards and initial certification of organic carp and tench production in Southern Germany using traditional pond culture techniques.

At the beginning of the new millenium, while the organic food market was still a niche market in terms of volume, it already offered most types of food in organic quality – with the exception of fish. Therefore, it was only a matter of time before this gap was filled. The development of organic carp production took place without great public attention on the farms usually with less than one hectare pond surface, typically run on a part time basis. Mostly this was due to the fact that originally the product was exclusively sold regionally in farmers markets or directly in farm stores. In total, there were 42 and 26 organic carp farming facilities operating in Europe and Asia, respectively, in 2009, however 7 and 22, respectively, were envisaged as new projects (Anonymous, 2010).

In the UK, the market for organic carp is considered still embryonic. However, the level of consumers' interest in sustainably farmed fish is encouraged by rising tradition of eating course fish in Europe because of consumer trust in organic standards. In the UK, the main potential outlets include restaurants, fish mongers, farm shops and box schemes. Adding value through processing (such as smoking) could further enhance returns. An exciting alternative to table fish is to grow carp (or perhaps other course fish) as a feed supplement for organic poultry or pigs, potentially providing a much needed source of protein. The organic carp farm at Upper Hayne Farm, near Cullompton in Devon, was established in July 2006, selling its first organic certified stock in November 2008. Currently, it manages 13 organic carp

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ponds covering about 2 ha (Hepburn 2009). The market for carp is considered as increasing annually in the UK, fuelled partly by Eastern European immigrants but also by more cosmopolitan tastes of British consumers. E.g., carp sale in Waitrose stores at Christmas increased by 50% between 2007 and 2008 (Lidell 2008).

Carp pond systems are self-sustaining, with little or no need for external inputs. Where organic fertilisation is needed, a maximum nutrient input of 20 kg nitrogen per hectare is applicable. Meanwhile, the new aquaculture rules explicitly prohibit chemical treatments for hygiene. Treatments involving synthetic chemicals for the control of hydrophytes and plant coverage present in production waters are prohibited. Carp ponds do not necessarily need water inflow once they are filled up, except to replace losses. Water loss would usually only occur through evaporation, which in central Europe accounts for an average loss of 1 litre per second per hectare, so it can be calculated that organic carp ponds produce up to 1,500 kg of fish biomass with only one litre per second of steady water supply.

Fish in carp ponds feed on naturally available food resources. The common carp itself and most cyprinids are omnivorous species, and feed on a mixed diet of plants, detritus and small animals (insects, worms, zooplankton...). Even if predator species are present, they do not receive external feed inputs. Supplemental feeding with organic crops, legumes and oil seeds is done where natural feed resources are not available in sufficient quantities; documentation is needed for such cases. Growth promoters and synthetic amino-acids are not allowed. Animal derived feedstuffs such as fish meal and fish oil are not appropriate for the species in carp pond systems (Anonymous, 2010).

Besides these requirements, (Censkowsky 2010) underlined that basic conditions of organic carp farming include also natural soil in ponds and organic origin of juvenile fish in minimum 20, 50 and 0% proportion in 2011, 2013 and 2015, respectively. Examples of successful organic carp projects mentioned were Biofisch (AT), Oberlausitzer Biokarpfen (DE) and Biohalak (HU). Price premiums for organic carp were 38, 30 and 40 % in 2006, 2007 and 2008, respectively; whilst for organic seafood in total it was 31, 29 and 32% in these years, respectively.

The main constraints and problems in organic carp production according to Varadi (2005) are:

- Shortage of organic feed
- Predation of wild (protected) animals
- Differentiation from non-organic
- Lack of cooperation on technical issues and marketing
- Intermuscular bones
- Consumer's perception of carp as a cheap food.

Organic carp processing into the form of boneless filets might be a good solution to support its marketing.

Organic carp farming standards try to minimize the impacts of pests, parasites and other factors negatively affecting fish health through the design of production system and husbandry practices, density limits, provisions for optimal feeding and the encouragement of production in polycultures. In their animal health management plan, operators must address biosecurity and disease prevention practices. Where veterinary treatment becomes necessary, non allopathic

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treatments are clearly preferred. In cases of reported disease (this must be well documented), medical treatments of any kind may be given within the standard organic framework, as long as the withdrawal period for allopathic veterinary and parasite treatments is observed. In addition, the use of allopathic treatments is limited to two courses of treatment per year – and if exceeded, those fish may not be sold as organic products. Generally, carp are considered as one of the examples of species well suited to self-sufficient polycultures under conditions of organic farming (Anonymous, 2010).

The way fish are farmed in ponds is already quasi-organic and the shift to certified organic farming of carp is not as demanding as it is for some other species but it takes time. The shift to organic farming includes a new feeding regime under which the fish are expected to nourish themselves using the naturally occurring food in the pond. Any additional feed that is given must be certified organic itself. In organic farming it is not permitted to have species that are propagated artificially so the Complex Group (Romania), oriented on carp organic farming, is gradually reducing the stock of Chinese carps (Anonymous 2013). However, certain confusions regarding organic carp also appeared with respect to the necessity of certification (Varadi. Phuong 2007):

- Why should carp be certified, - they are more or less organic anyway
- Is it worthy to pay for organic?
- If the product is not organic, does it mean that it has a lower quality?

Organic carp farming is not only the recent issue of modern society – e.g. Apatani, a hill tribe of Arunachal Pradesh, India, has been practising „organic“ rice-fish culture traditionally for many decades. Their practice, locally termed „aji ngui assonii“, is free from the use of agro-chemicals and additional input of supplementary feed for fish. They basically follow the traditional agronomic practices for rice even in rice-fish combination pertaining to field preparation and maintenance. The strains of common carp are stocked at fry stage (3-5 cm), after just ten days of planting the rice and fish are reared in the field for about 4 months in total. Sometimes they harvest fish partially from the field after an interval of 1.5 months. With multiple harvesting, only 200-300 kg/ha of fish are produced in each harvest whereas with final harvesting the production rate was 500 kg/ha. The system of such integrated farming is an organic practice, as well as sustainable in the sense that it is based solely on available natural resources in the ecosystem and also preserves the agro-biodiversity, enhancing multidimensional support to the tribal livelihood (Osoli, Taleshi 2010).

As proved by the monitoring of Austrian organic and Czech conventional carp farming ponds (Anton-Pardo et al. 2014), their environmental variables presented a high variability but no differences between the two ways of carp production. The same was true also for zooplankton density which showed a wide range of values but insignificant differences. With few exceptions, rotifers were the most abundant group of zooplankton in the ponds under study. Despite no significant differences in cladoceran density, the abundance of large caldocerans (*Daphnia* spp.) was lower in organic ponds.

Among carps, organic farming does not concern exclusively the common carp. As reported by Majhi et al. (2006), the aquatic fern azolla (*Azolla caroliniana*) can be easily grown organically in North Eastern India due to favorable climatic conditions. This fern was successfully fed to the grass carp, *Ctenopharyngodon idella* (Val.) as a step towards producing organic fish. Azolla was found to be a preferred feed by the grass carp and mean daily growth increment

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was statistically proved to support the final weight gain of grass carp in azolla fed ponds compared to that in the control pond. The net profit for production of organic grass carp was calculated as 0.12 \$/m². It was concluded that the utilization of organic azolla through grass carp is one of the best options for production of fish biomass from the aquatic habitat. This would also pave way for producing organic fish and help in bridging the gap between production and demand for organic fish in India.

Chemical composition and caloric value of organic common carp, bighead carp (*Aristichthys nobilis*) and grass carp meat, was influenced by dietary fat contents. The body fat level was higher in bighead carp meat compared to carp and grass carp meat, grown in ponds, where organic fertilization has been applied. The proteins share in the fish meat showed a more clearly expressed differentiation depending on the species and ranged within 77.0-79.6% for bighead carp, 81.3% for carp and 83.5-84.5% for grass carp (Hadjinikolova et al. 2008).

Current organic standards for common carp are facing some inconsistencies such as for propagation. Shortage of organic feed, predation of wild animals, differentiation from non-organic product, lack of cooperation on technical issues and marketing, bones appearance and consumer perception of carp as a cheap food make the main constraints for organic carp farming and marketing. The margins for organic carp can be good with sales price being significantly higher than production costs. It is suggested to consider carp as an ideal candidate for organic culture due to easy to convert farms. Organic carp culture needs help to harmonise standards, to improve processing and marketing (boneless fillets) and to support greater cooperation among organic farmers. Organic carp products show a potential in existing organic market e.g. in Germany and Austria and will benefit the whole sector. There are good long term prospects with this respect, though current consumption is on the wane. There is a need to reposition carp in the market and produce more fillets rather than just whole fish. The standards and differences regarding the use of hormones should be harmonised (Anonymous, 2005). Currently, the key issues are lack of stock fish of organic origin (Marc Mössmer, pers.comm.), processing, market organisation and market communication (Varadi, Phuong 2007).

A1.3 Other species

A1.3.1 Shellfish

Shellfish (molluscs) are extracting nutrients from the natural local feed web and included in compensation farming; i.e. farmed adjacent to cage farming of fish and then absorbing nutrients discharged from the fish to minimize the environmental impact from cage fish farming.

Conventional shellfish aquaculture is often promoted as an example of sustainable aquaculture (Shumway et al., 2003). However, more detailed rules are required to be able to separate more clearly conventional from organic shellfish aquaculture.

Related to Article 15 (e) (ii) in Regulation 834/2007, growing areas for organic aquaculture are waters classified as Class A (direct human consumption) or Class B (human consumption after treatment in purification centre). Organic shellfish aquaculture should be limited to those areas where the harvested product can be directly used for human consumption (Class A waters).

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This step could also be taken as a way to further differentiate conventional from organic aquaculture.

There is a lack of control over the quality of the natural feed webs present in areas with shellfish aquaculture (annual fluctuations in the composition of shellfish feed are also not considered). Hence, adequate monitoring programs should be established in areas of organic production, cf. Regulation 834/2007, Article 15 (e) (iii).

More specification is required regarding the culture density allowed in organic mollusc aquaculture. Currently the regulation only indicates that stocking density should not exceed that used for non-organic shellfish culture in the area, cf. Regulation 710/2009, Section 6; Article 25p (1).

From 1st January 2016 seed shall be produced in organic shellfish hatcheries. However, organic hatcheries may not be established in due time due to the high costs required for establishment of organic shellfish hatcheries/nurseries, c.f. Regulation 710/2009, Section 6; Article 25o (1).

A1.4 Recommendations to the regulation - 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. Fishmeal 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 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.
- Until more knowledge is available fish meal and fish oil derived from industrial fish, not used for human consumption and 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 shrimp diets 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.

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- If not available from organic procedures, essential amino acids and lipids obtained by fermentation or other similar procedures 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 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 as well as fish health aspects of marine oils (HUFAs) in diet.
- Adjust regulation on request of exchanging fish oil by vegetable oils in accordance to development of vegetable or other sources providing omega-3 fatty acids (HUFAs).
- Prioritize research in alternative sources of Omega-3 fatty acids (HUFAs).
- Chemically well-defined analogic substances of minerals and vitamins should be considered for use if the natural substances are unavailable.

A2.0 Welfare, Health, Veterinary treatments and Biosecurity

A2.1 Welfare

A2.1.1 Introduction: Present regulations

According 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 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 (T-AP)."

According to EC Reg. 834/2007, art. 15 1(b)(ii), (EU, 2007): "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;"

According to EC Reg. 889/2008, art. 25f 1/2/3/4/5, (EU, 2008): "Aquaculture Husbandry practices. General aquaculture husbandry rules.

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

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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.

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.

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.

4. Containment systems shall be designed, located and operated to minimize the risk of escape incidents.

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.

According to EC Reg. 889/2008, Art. 25h 1,2,3,4, (EU, 2008): Management of aquaculture animals.

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.

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.

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.

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.

Further EC Reg. 889/2008, Annex XIIIa (EU, 2008) on production systems and stocking density.

In aquaculture many kinds of fish production systems such as ponds, tanks, open and recirculation systems, silos and cages are employed. Each of them are different in terms of water quantity, quality, temperature, etc... Water is the medium in which farmed fish 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 themselves.

Water quality is often characterized by chemical parameters such 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).

A2.1.2 State of the art

Fish welfare represents an important issue for the aquaculture industry from both a practical and ethical point of view. From a practical point of view, production efficiency, quality and quantity are often coupled with good welfare. Additionally, the public is increasingly concerned about the welfare of farmed fish, highlighting the ethical significance of fish welfare (Ashley, 2007). As a result, fish welfare has become a growing area of research (Ashley, 2007), in an attempt to develop husbandry techniques that promote welfare in farmed fish (Huntingford and Kadri, 2009). Animal welfare is generally referred to as the physical and mental state of the animal interacting with its environment and associated variations (Chandroo et al., 2004).

The magnitude of an animal welfare problem may be evaluated as the product of the *severity of suffering*, its *duration* and the *numbers of animals affected* (Mood & Brooke, 2012).

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, and sub-optimal stocking densities and social environments (Ashley, 2007; Huntingford et al., 2006).

Welfare may be related to the conditions that consider an animal to be in a state of good welfare: 1) Feelings-based, 2) Nature-based and 3) Function-based (Huntingford and Kadri, 2008). 'Function-based' definitions basically assume that welfare is correlated with biological functioning, including physiological stress responses (Duncan 2005), while in the 'Feeling-based' definitions, 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). In addition, it is increasingly clear that individually 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 and aggression are favourably linked to management that follow and develop routines.

1) When using feelings-based definition of welfare we will categorize 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, 2009; Huntingford and Kadri, 2008). This implies that even though an animal is healthy, it may not necessarily experience good welfare unless this generates positive feelings. Likewise, an animal that is injured may not necessarily experience bad welfare unless this generates negative feelings (Huntingford and Kadri, 2008).

2) By using nature-based definitions we will categorize 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). These definitions imply that if animals can show their natural behaviour they experience good welfare and if they cannot express their natural behaviour they experience bad welfare (Huntingford and Kadri, 2008).

3) Function-based definitions identify an animal to be in a state of good welfare if the animal “...can adapt to its environment and is in good health, with all its biological systems working appropriately...” (Huntingford and Kadri, 2008). These definitions imply that if an animal is in good health and has proper functioning of bodily systems, they are experiencing good welfare. On the other hand, if an animal has bad health and has impairment to bodily functioning, they are experiencing bad welfare (Huntingford and Kadri, 2008).

A2.1.2.1 Stress

Stress is a central issue in fish welfare as activation of the stress response in fish has been demonstrated to have potential maladaptive consequences affecting welfare (Huntingford et al., 2006). Indicators of the physiological stress response are therefore commonly used in welfare studies (Ashley, 2007).

The stress response in fish is initiated by a series of neuroendocrine events that function to re-establish homeostasis when confronted with an environmental challenge (Ashley, 2007; Ellis et al., 2012; Pottinger, 2008).

A2.1.2.2 Stocking density

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. However, 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).

Furthermore, this relationship between rearing density and welfare is often variable between studies, attributed to the study specific nature of each study, indicating the specific environmental conditions (oxygen, waste removal capacity) act to influence how stocking density affects welfare (Ellis et al., 2002). As such, 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).

Boujard et al. (2002) investigated the effect fish held at stocking densities of 25, 70 and 100 kg/m³ 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 (Boujard et al., 2002)

North et al. (2006) studied the impact of the stocking densities of 10, 40 and 80 kg/m³ on a variety of physiological and morphometric indicators. They demonstrated that being held at high density (80 kg/m³) 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/m³). 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/m³) 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 m³) compared to low density (45 kg m³), but that fin condition was improved at high density (Rasmussen et al., 2007).

The combined effects of stocking density (25, 74 and 120 kg m³) 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. However, irrespective of water quality, growth performance was the lowest at the high density (120 kg m³) although no major physiological disturbances were observed (Person-Le Ruyet et al., 2008).

There are two studies that have investigated the combined effects of stocking density (~ 25 and ~ 100 kg m³) and sustained exercise (water current of 0.9 bl s⁻¹) in trout. 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).

Hosfeld et al., (2009) reported that densities of up to 86 kg/m³ of Atlantic Salmon parr had no detrimental effect on growth, condition factor or overall plasma glucose levels in a 100 day study.

Brockmark et al., (2007) reported an experiment on very low stocking densities of Atlantic salmon pre-smolts (1.35 kg/m³ vs 3.75 kg/m³). Fish in low density tanks grew better, had better condition factor, less fin damage and lower mortality rate than those at higher densities.

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Cañon Jones et al., (2011) compared salmon parr at 8 kg/m³ or 30 kg/m³. Fish grew better, had better condition factor and had lower total aggressive interactions (attacks, displacements and fin bites) at 30 kg/m³. However, fish at 30 kg/m³ had significantly more overt aggression (fin bites) and fin damage than fish at 8 kg/m³.

For Atlantic salmon during on-growing in seawater Kjartansson et al., (1988) found that densities of 100-125 kg/m³ had no significant effect upon growth or condition factor throughout the study compared to 35-45 kg/m³. However, at day 101 haematocrit, glucose and lactate were significantly higher in fish stocked in densities of 100-125 kg/m³ compared to 35-45 kg/m³.

Adams et al., (2007) looked at 15, 25 or 35 kg/m³ densities of seawater reared salmon. Overall, welfare was highest at 25 kg/m³ and lowest at both 15 and 35 kg/m³.

Oppedal et al., (2011) compared normal (5.6–14.5 kg/m³) and high (15.7–32.1 kg/m³) stocking densities of salmon in cages. Fish under normal density showed better growth, condition factor and better feed intake. When stocking density reached level of 25–30 kg/m³ there was an increase in fin damage and lesions.

Turnbull et al., 2008 reported that a density of over 22kg/m³ was a risk to welfare of salmon.

The literature is also conflicting on optimum stocking densities for tank-held adult salmon in seawater.

For sea bream 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 for 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, demonstrating that substrate and density effects are socially-induced.

Farmed fish experience un-natural high densities within the cages, which have also been related to increased of plasma cortisol and compromised fish growth and welfare. Increased stocking density, resulted also in 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 at higher densities. Additionally, sea bream was further attracted by damages on the net pens, while individuals were capable of extending existing damages. In particular, fish fed restricted diets were more attracted by damages on the aquaculture net structure and showed 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 high 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).

Sea bass (adult and juveniles) reared at high density exhibited slower growth than under low rearing densities (Saillant et al., 2003, D'Orbcastel et al., 2010). There is, anyway, some

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discordance about density effects on fish growth. Lupatsch et al. (2010) didn't 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 increased red muscle activity leading to a rise of the global scope for activity (Lembo et al., 2007). At 50 kg/m³, fish muscle activity, measured as EMG activity with telemetric technologies, was on average twofold higher than in fish at 10 kg/m³ (Carbonara et al., 2013).

The overall picture arising from the studies in fresh water 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 salmonids. 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 effects of stocking density on fish welfare, and the effects of several other 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.

A number of the reviewed studies have attempted to make specific recommendations for maximum rearing densities. Clearly, wide discrepancies exist with regard to the maximum stocking density recommendations and the recommendations for optimal stocking from a welfare perspective remain elusive. Such a wide range of recommendations highlights the fact that it has been a challenge to identify density limits that promote optimal welfare and fish production. This is in part due to a lack of understanding of how the different environmental factors interact with each other and with stocking density to affect welfare (Ashley, 2007). Another reason maybe 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.

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 is evidence for the formation of dominance hierarchies in rainbow trout held at low densities (North et al., 2006).

It is commonly assumed that in salmonids, aggression decreases with increasing density. This maybe 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 schooling behaviour in salmonids (Grand and

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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 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).

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 challenging task for the Danish authorities (Larsen, 2014).

The challenge was to translate occurring 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. F. ex. so was the case for the rules of transport in art. 32a (3): "Precautions shall be taken to reduce stress", which have raised several questions on how specifically to interpret this rule (Larsen, 2014).

Based on interpretation of the current regulation EC Reg. 710/2009, (EU, 2009) the Danish authorities on inspection of organic aquaculture facilities has prepared a kind of check-lists for approving and control of organic production facilities (Larsen, 2014).

The list regarding stocking densities included (Larsen, 2014):

Salmonids in freshwater (FW): Open systems – do not exclude recirculation.

- Min. 60 % oxygen saturation
- Salmon, arctic char: max. 20 kg/m³
- Sea- and rainbow trout: 25 kg/m³

Salmonids in seawater (SW):

- Salmon, sea- and rainbow trout: Max. 10 kg/m³ in net cages

Cod, bass, bream, turbot, ..in sea and on land: Production in cages at sea or in open land-based facilities with sufficient water current for optimum welfare.

- Turbot: 25 kg/m²
- Others: 15 kg/m³

Bass, grey mullet, eel in earth ponds in tidal zones or coastal lagoons: Secure adequate renewal of water for welfare. 50 % of dikes must have plant cover. Wetland based depuration ponds required.

- Max. 4 kg/m³

According to animal welfare and health considerations, the organic aquaculture implementing rules limit annual biomass gain of carp to a maximum harvest of 1,500 kg per hectare per year, or a maximum density of 1.5 kg per m³. Biodiversity requirements for the aquatic ecosystem and the vegetation in and around production systems are both relevant to carp pond operators and should be easy to fulfil.

A2.1.2.3 Water quality

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, elevating plasma cortisol levels (Pickering et al., 1991). The threshold oxygen concentration for growth in rainbow trout has been shown to be ~ 75 % saturation (Pedersen, 1987).

Sea bream is more sensitive to hypoxia than sea bass. Oxygen concentration of 5 mg/L 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).

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 increased dissolved oxygen (DO), both due to reduced water temperature and to the mild oxygen supersaturation following application of pure oxygen. The advantage for fish may be found in the compromise between maximising O₂ diffusion at the gills and ions/water intake/loss, known as “osmoregulatory compromise”.

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 O₂ kg⁻¹h⁻¹, is between 10 and 25°C. Indeed, also the significant interaction between temperature and oxygen concentration suggests that the higher the temperature, the greater the energetic expenditure (Claireaux and Lagardère, 1999).

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

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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 *S. aurata* 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% (Wajsbrodt et al., 1991).

Water temperature is also an important physical factor for welfare of aquatic organisms, e.g. sea bream borne in the open sea during October-December. Juveniles typically migrate in early spring towards protected coastal waters, where they can find abundant trophic resources and milder temperatures. The species is very sensitive to low temperatures (lower lethal limit is 4°C). 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 O₂ 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 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.

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Evidences were reported by Barnabé (1991) who observed that sea bass juveniles ceased growing at 11-15°C and grew 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 showed 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).

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.

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).

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 O₂ concentration acting as limiting factors. In sea bass, fins result 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 that, 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 lead both to lower fin damages as well as to lower growth rates.

Salinity plays a significant role in both salmonids and in the euryhaline sea bream and sea bass, whose juvenile appears in low salinity lagoons and river estuaries. Sea bream 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 was observed a "U-shaped" relationship 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 hr 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 cases of acclimation of gilthead sea bream is also reflected by the relative modulation of the immune system. Indeed, acclimation to a wide range of salinity from low saline water (LSW, 6 p.p.t.) to hypersaline water (HSW, 55 p.p.t.) 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).

The sea bass capabilities to adapt and live in 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 h 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.

A2.1.2.4 Behavioural interactions

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). Using physical enrichment materials e.g. by providing substrate, or providing shelters to increase habitat complexity can improve welfare by reducing aggression (Batzina & Karakatsouli, 2012; Batzina et al., 2014) and reducing injuries (Persson & Alanära 2014).

A2.1.2.5 Light regime requirements

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 (2008a) 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

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use of light is an efficient way of reducing the welfare problems associated with salmon maturing in sea-water.”

The effect of photoperiod manipulation for organic salmon farming, both for the freshwater and seawater phases of production is un-known. Robust data on the welfare effects of extending daylength are scarce, but the data that is available, does not suggest high risks to welfare aside from potential appetite suppression in post-smolts (Oppedal et al., 2003). These risks may also be ameliorated by choosing lighting technologies that have more appropriate spectral composition and intensity (Migaud et al., 2007).

There is 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.

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).

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.

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).

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.

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A2.1.3 Ethics

A2.1.3.1 Introduction

Ethical consideration of fish can be based on 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 in combination with, respecting individual fish as sentient beings, much in parallel to arguments for respecting dogs and pigs.

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 (without ranking) e.g. fair labour conditions, economic viability in small and large scale production systems, respectively. Further, the 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. 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 (Braithwaite et al., 2013, Braithwaite 2010, Chandroo 2004; Colin 2013, EFSA 2009).

Having a revision of EU regulation for organic aquaculture in mind, an important to refer to the Lisbon Treaty (EC 2007) which states that "In formulating and implementing the Union's

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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) is more or less based on fish being 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 (Röcklinsberg 2014).

Of further relevance for revision of the 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 reflected in the views expressed by some stakeholders (WP1).

In descriptive ethics empirical studies are necessary to present and describe what is actually done in a certain context. Such studies make no claims on evaluating if certain behaviour is better or fairer than another. In normative ethics, on the other hand, the aim of empirical studies may be to enhance context-sensitivity (Musschenga 2005) and to bridge the gap between empirical facts and normative statements (Molewijk et al 2004). In the present study the idea was 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. In case a normative statement is requested, knowledge of praxis contributes to well based decisions (Röcklinsberg 2001). Hence an ethical approach to empirical studies on fish wellbeing might open for a range of perspectives.

A2.1.3.2 Welfare

For animal welfare natural behaviour is 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 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 relate welfare to biological functioning/health issues e.g. in terms of stress (cortisol) measurement, osmoregulation, fin erosion or body injury, suppressed growth rate, elevated metabolic rates, disease and mortality (Ellis et al 2002), none 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 (tailbeat frequency) and iv) overt erratic behaviours, and also in the paper by Alänärä and Brännäs 1996.

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Although some consumers tend to think that fish is a “semi-animal”, half a vegetable (Kupsala et al., 2013), increased interest for fish welfare can be seen in Europe (Huntingford et al 2006). Further, the organic farmer has to fulfil several check points, reflected in the regulation and controlled by the certifying body to be certified as organic producers, However, if an aim with revision of organic regulation would be to widen the gap to standards for conventional fish farming, behavioural studies on all farmed species would be most valuable, as well as education and training of employees to secure welfare during transport and handling practices. However, confer EC Reg. 834/2007, recital (17), (EU, 2007) stating: “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”.

Given fish welfare would be considered important by a large part of society in a near future, 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 (maximize the positive consequences for as many (sentient) individuals as possible) organic aquaculture could argue for taking small steps in order to include as many individual fish as possible, i.e. reach as many producers as possible. How to pay full attention (as claimed in the Treaty) to animal welfare and take the responsibility of knowledge of fish capacities, IFOAM's principles of naturalness, care and health, and legislator's claim to pay full regard to the welfare requirements of animals serious? Or, in the formulation of EC Reg. 834/2007, Art. 15 1(b)(vi) re Production rules for aquaculture animals: “any suffering of the animals including the time of slaughtering shall be kept to a minimum”.

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).

One step in the production process where unnecessary suffering could occur, but also is proven possible to improve given it is not defined in terms human ends justifies all animal suffering is the last phase in the fish life, crowding in tightened nets and the pumping to the slaughter house. 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 CO₂, as this has proven to be inefficient and to cause stress/suffering.

Another question is the implementation and interpretation of the current legislation – is there room for a wide range of interpretations, or is any of these - in a strict sense - illegal? The aim

of the organic regulation was a need for harmonisation of legislation 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 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 higher level of harmonisation of Organic Aquaculture gain those countries having a ‘stricter than necessary’ interpretation and those ‘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?

A2.1.3.3 Stocking density

It is obvious there are quite some difficulties related to assessment of welfare, not only due to species, large variation in transport and farming conditions, but also in the assessment of welfare. What to measure – mainly external aspects such as water quality and temperature, feeding method, rearing system and environmental conditions or also internal biological reactions by the fish (Ashley 2007). In both cases results are to be interpreted and compared with something else, and it has proven difficult to find a common scale. From an ethical 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 discrepancies 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. On the other hand, it might be hard because economic aspects are implicitly taken into account.

If arguing that natural behaviour is important for the individual fish welfare, a wider range of issues need to be included and related to each species concerned - from reproduction behaviour, to feed and to 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. This issue relates back to the above mentioned issues; consumer perspectives and perception on animal welfare, level of harmonisation between organic standards, as well as choice of level of difference between organic and conventional regulations.

A2.1.3.4 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; i.e. “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 stunning (electricity 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). Certain levels of training and education might be considered in the Organic regulation.

A2.1.4 Recommendations to the regulations - Knowledge gaps

- Data on optimal stocking densities are conflicting. More studies of the effects of density on water quality and a multitude of operational behavioural, physiological and morphological welfare indicators are needed to consider robust limits for stocking density in organic aquaculture
- The potential benefits of providing fish with access to nature-like substrates are species specific. More data needed on type of substrates for specific species. Current knowledge suggests e.g. salmonids and maybe other sp. may not have a preference for substrate *per se*, but a preference for shelter, be it overhead, floating or benthic
- More knowledge is needed on the significance of light regimes requirements on the welfare and performance in organic aquaculture.

A2.2 Transport

A2.2.1 Introduction: Present regulations

According to EC Reg. 834/2007, Art. 15 1(b)(v), (EU, 2007), "transport shall ensure that the welfare of animals is maintained".

According to EC Reg. 889/2008, Art. 25h 4, (EU, 2008), "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."

'According to EC Reg. 710/2009, Art. 32a (EU, 2009), "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.

A2.2.2 State of the art

Live fish shall be transported in adequate tanks using clean water, i.e. not system water, fulfilling the physiological needs of the fish with respect to oxygen, i.e. 65 – 120 % saturation and temperature, i.e. 0 - 20 °C. The temperature of the water in the transport basins should have the same temperature as the water 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. This to minimize metabolism and production of metabolites (NH₄⁺) and particulate matter, that will deteriorate water quality, deposits in the gills and further stress the fish (Larsen, 2014).

The tanks shall be carefully cleaned, disinfected and flushed before used for transportation of organic fish and fish products. Stress shall be minimized, i.e. max. stocking density 150 kg/m³ 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 hours. Total retention

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time in transportation tanks and concomittant storage in tanks at the slaughtery should not exceed 24 hours.

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, i.e. other conventional farms, and always using a route, which could be approved for organic farming.

Documentation for all activities shall be available.

Transport of juvenile salmon in freshwater showed that Loading process appeared to be more severe a stressor than transport (Iversen et al., 2005; Nomura et al., 2009).

Iversen and Eliassen, 2009 and Iversen et al., (2009) found reduced stress during transport of salmon smolts treated with Isoeugenol and clove oil, respectively.

Experiments with seawater open-hold transport of post-smolts and adult salmon have shown, that good water quality was the most important at short term transport (1-2 hrs) of 25 t salmon (5 kg) at 125 kg/m³ (Erikson et al., 1997; Farrell, 2006; Gatica et al., 2010; Nomura et al., 2009; Tang et al., 2009a). The most stressfull was the pumping process.

Experiments with seawater closed-hold transport of post-smolts and adult 5 kg salmon 135 kg/m³, 10 °C in wellboat showed, that CO₂ accumulated during the transport at sea (5 mg/l). However it was well below threshold value and did not compromise welfare (Tang et al., (2009b).

Methods to alleviate exposure to stressors (need of oxygen and reduce ammonia production) during transport include cooling of the water (Lines and Spence, 2012).

However, Freedom Foods Welfare Standards 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 24h, and iii) minimum temperatures at the end of chilling should be no less than 4°C.

By killing organic fish they shall be made unconscious as gentle as possible and immediately after been taken out of the water, i.e knock at the head.

A2.2.3 Recommendations to the regulations - Knowledge gaps

- For transport of farmed salmon the oxygen content in the water should be at least 7 mg oxygen per litre
- Excessive changes in water temperature and pH during transportation must be avoided
- Smolt densities of up to 70 kg/m³ by road transport for up to 90 minutes did not compromise fish welfare
- Open-hold wellboat transport, densities of up to 150 kg/m³ for >10 hours had no significant effect upon salmon welfare
- Max. density with transportation of fry could be set to 10 kg/m³
- The loading phase appears to be more detrimental to welfare than the transport phase and well boat transports seemed to have an important recovery function.

Knowledge gaps:

The effects of isoeugenol on large scale transport of smolts are not well investigated

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- The potential welfare costs/benefits of large scale live chilling during transport need to be investigated in greater detail for adult fish.

A2.3 Killing - Slaughter

A2.3.1 Introduction - Present regulations

According to EC Reg. 834/2007, Art. 15 1(b)(vi) re: Production rules for aquaculture animals: "any suffering of the animals including the time of slaughtering shall be kept to a minimum;" (EU, 2007).

According to EC Reg. 889/2008, art. 25h 5, re.: Management of aquaculture animals: "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." (EU, 2008).

A2.3.2 State of the art

Fish slaughter is the process of killing fish, typically after harvesting at sea or from fish farms. Some relatively humane slaughter methods have been developed, including percussive and electric stunning. However, methods like suffocation in air, carbon-dioxide stunning, or ice chilling are still practiced, but are widely regarded as inhumane.

Research on fish suffering during slaughter relies on measures to indicate when fish are conscious and experiencing pain. Some indicators used by welfare studies include: Behavioral swimming, gill movement, eye movement in response to body re-orientation, reaction when inverted, etc. (Poli et al., 2005)

Following electric stunning, as fish gradually resume consciousness, they begin to make rhythmic gill-cover movements. Based on EEG correlations, it is believed that stunned fish remain insensible until they have resumed rhythmic gill patterns (Kestin et al., 1995, 2002). This can be used as a convenient assessment tool for the effectiveness of electric stunning (Lines et al., 2003; Lines and Spence, 2012).

In 2004, the European Food Safety Authority observed that "Many existing commercial killing methods expose fish to substantial suffering over a prolonged period of time." (ESFA, 2004; 2009; Lowe et al., 1993). Hence, according to the Aquatic Animal Health Code of the World Organisation for Animal Health the following slaughter methods are considered inhumane.

Starvation of the fish before slaughter (1 – 5 days dependant on temperature, fish size) is commonly performed to empty the gut and to reduce the probability of fish being contaminated with feed and faeces during the subsequent slaughter procedure. This practice also induces a reduction in ammonia excretion by the fish, thus reducing the water quality deterioration occurring during, crowding and transport to slaughter facilities (Algers et al., 2009; Einen and Thomassen 1998; Rasmussen et al. 2000). However, prolong starvation may lead to immune depression, which makes fish more susceptible to stress mediated diseases during the pre-slaughter period (Algers et al., 2009). Further prolonged starvation may lead to shorter shelf-life of the product (Álvarez et al., 2008).

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 and pre-slaughter procedures (Lowe et al., 1993; Nakayama et al., 1999; Bagni et al., 2007). Bagni et

al. (2007) reported that rigor starts earlier in crowded fish. Indeed, the post mortem catabolism varied considerably between stressed and unstressed fish, where ATP is more or less depleted, respectively (Berg et al., 1997).

Air asphyxiation

This is the oldest slaughter method for fish and is considered inhumane because it can take the fish over an hour to die. Meat quality and shelf-life are also diminished (Poli, 2005).

Ice bath

Also called live chilling, this method involves putting fish in baths of ice water, where they chill and eventually die of anoxia. Because chilling slows metabolic rate and oxygen needs, it actually may prolong the duration until death, i.e. more than an hour (Poli, 2005).

CO₂ narcosis

Most often applied for salmon and trout, CO₂ narcosis involves filling the fish water with CO₂ to produce acidic pH, which injures the brain. The procedure is apparently painful, as evidenced by fish swimming vigorously and trying to escape from the tank. CO₂ immobilizes the fish within 2–4 minutes, but the fish remain conscious until subsequent stunning or killing. CO₂ stunning has at least been banned in Norway since 2012 (Kleingeld, 2013; Chilvers, 2014).

However, the following slaughter methods are considered more humane.

Percussive stunning

Percussive stunning means knocking the head of the fish with a wooden or plastic club. One or two appropriate blows can disrupt the brain sufficiently to render the fish insensible and potentially even kill it directly. However, applying this method correctly requires training and effort. Percussive stunning must be applied one fish at a time and so is typically only used for large fish, such as salmon and trout. If the operator is skilled, percussive stunning can be among the most humane methods and can also yield high meat quality. One comparison of slaughter methods found that percussive stunning had the best welfare performance as measured by low hematocrit, low plasma glucose, low lactate, and high muscle energy charge (Kleingeld, 2013, Van de Vis et al., 2003, Terlouw, et al., 2008, Roth et al., 2012).

Electrical stunning

Electricity can be relatively humane if applied correctly. The majority of the Norwegian fish-slaughter facilities has switched to either percussive or electrical stunning (Fishcount, 2014).

Spiking

Spiking is a slaughter method where a sharp spike is stuck through the brain of the fish. If done properly, it can kill quickly; however, if the operator misses the brain, the results may be traumatic for the fish. As with percussive stunning, spiking is used to kill one fish at a time and so is mainly used for large fish.

Handling of fish immediately prior to slaughter

Crowding

Prior to slaughter (normally 1-3 days) e.g. salmon are transported with well-boats from the on-growing farm to the slaughter house where they are placed in waiting cages close to the slaughter house. To facilitate pumping to the slaughter line the fish are crowded by tightening the net so density may be up

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to 300 kg/m³. This may accomplish increased behavioural panic, lactate and cortisol (Espmark 2005), injuries, increased stress and reduced welfare (e.g. Skjervold et al 2001, Roth et al 2012; Southgate and Wall, 2001).

Water quality

Keeping good water quality is a critical issue, which also might compromise fish welfare during storage in waiting cages prior to slaughter (Espmark et al 2012).

Pumping: Pumping by vacuum 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).

In a report from EFSA (2009a) it was 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.

A2.3.3 Recommendations to the regulations - Knowledge gaps

- When properly done the most humane stunning methods is percussive and electric stunning. The methods should be followed by killing with gill cut.
- Throughout storage prior to slaughter water quality should be monitored and continuously adjusted accordingly
- Use properly pump equipment with care and managed by trained staff
- Personnel should be regularly (annually) trained regarding fish welfare and equipment
- Alternatives to waiting cages should be investigated

Knowledge gaps:

- There are alternative stunning methods that are too poorly investigated to evaluate them regarding humane slaughter (e.g. CO₂, alternative anaesthetics)
- The use of electric stunning is considered as humane, but today the method is complicated and not user friendly and not easy for commercial application
- Waiting cages should be avoided, but so far there are few realistic alternatives on the market.
- There are few documentations on water quality in waiting cages

A2.4 Health - Veterinary treatments - Biosecurity

A2.4.1 Introduction: Present regulations

According to EC Reg. 834/2007, rec. 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 (T-AP)." (EU, 2007).

According to EC Reg. 834/2007, art. 4 (a)(iii): " Exclude the use of GMOs and products produced from or by GMOs with the exception of veterinary medicinal products;" (EU, 2007).

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According to EC Reg. 834/2007, art. 5(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" (EU, 2007).

According to EC Reg. 834/2007, art. 15 1(f): "with regard to disease prevention and veterinary treatment: (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." (EU, 2007).

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

According to EC Reg. 889/2008, art. 25s (1/2/3/4/5/6): «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» (EU, 2008). 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. 6. For biological control of ectoparasites preference shall be given to the use of cleaner fish.

According to EC Reg. 889/2008, art. 25t 1/2/3/4: "Veterinary treatments: 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 homeopathic 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 can not 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 is not specified 48 hours” (EU, 2008).

According to EC Reg. 889/2008, Annex VII 2.1: Substances for cleaning and disinfection of equipment and facilities, in the absence of aquaculture animals and 2.2: Limited list of substances for use in the presence of aquaculture animals.

A2.4.2 State of the art

The development of non-antibiotic and environmentally friendly agents is one of the key factors for health management in aquaculture. Hence, the use of probiotics is a viable alternative for the inhibition of pathogens and disease control in aquaculture species. A bacterial supplement of a single or mixed culture of selected non-pathogenic bacterial strains is termed probiotics. Probiotic microorganisms have the ability to release chemical substances with bactericidal or bacteriostatic effect on pathogenic bacteria that are in the intestine of the host, thus constituting a barrier against the proliferation of opportunistic pathogens. In general, the antibacterial effect is due to one or more of the following factors: production of antibiotics, bacteriocins, siderophores, enzymes (lysozymes, proteases) and/or hydrogen peroxide, as well as alteration of the intestinal pH due to the generation of organic acids (Cruz et al., 2012). Traditionally, probiotics used in food industry have been deemed safe, in fact, no human risks have been determined, remaining as the best proof of its safety (Cruz et al., 2012).

A wide range of microalgae (*Tetraselmis*), yeast (*Phaffia* and *Saccharomyces*), gram-positive (*Bacillus*, *Lactococcus*, *Lactobacillus*, *Streptococcus*) and gram-negative bacteria (*Aeromonas*, *Pseudomonas* and *Vibrio*) have been evaluated as probiotics. Several microalgae, yeasts and gram-positive and-negative bacteria have been isolated from the aquatic medium.

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; Burbank, 2011).

The application of probiotics in aquaculture has been regarded as a sustainable and promising strategy not only in the context of disease control but also in nutrition, growth and immunity. Probiotics are good bacteria that confer beneficial actions to the host or to their environment through different modes of action. The science of probiotics has evolved through the years and its diversification is an adaptation to the growing number of host species utilizing these beneficial bacteria. Probiotics may play a significant role during the early stages of development in the host fish, in particular larvae of marine fish species (Lazado et al., 2014.).

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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 (Sommerset et al., 2005; Clarke et al., 2013).

The use of plants for vaccine production offers several advantages such as low cost, safety and easy scaling up. 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.

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.

Consideration might be focused on the utilization of plants for low cost and large quantity production of fish vaccines with oral immunization by plant genetic engineering, especially plastid genetic engineering of edible crops (Clarke et al., 2013).

The use of medicinal plants in aquaculture has attracted a lot of attention globally and has become a subject of active scientific investigations. Hence, the use of plant-derived products could represent a promising approach complementary to vaccination and traditional drugs; e.g.:

- Biologically active secondary metabolites (alkaloids, phenols, flavonoids, tannins, terpenes..)
- Growth enhancer, appetite stimulation, antimicrobial, immunostimulant, anti-inflammatory, anti-stress properties
- Easily available and biocompatible (Kolkovski & Kolkovski, 2011; Bulfon et al., 2013; Reverter et al., 2014.).

The most investigated herbs are those widely used in folk medicine in China, India, Thailand and Korea, such as *Angelica sinensis*, *Astragalus membranaceus*, *Echinacea purpurea*, *Solanum nigrum*.

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.

Hence, ginger is effective for the control of a range of bacterial, fungal and parasitic conditions. Also, ginger has been reported to have anti-inflammatory and anti-oxidative activity and to be effective as an immuno-modulatory agent in animals, including fish (Nya & Austin, 2009a).

Garlic, *Allium sativum*, has a history of dietary and medicinal applications as an anti-infective agent. Interest in garlic as an immunostimulant for application to aquaculture follows its use in human medicine and agriculture as a proven prophylactic and therapeutic agent.

Evidence of its value includes inhibition towards bacteria, fungi, viruses and protozoa.

In use, garlic leads to stimulation of immune functions in humans and in fish (Nya, B. Austin. 2009b; Harikrishnan et. al., 2011).

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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.), (Kolkovski & Kolkovski, 2011; Chakraborty & Hancz, 2011; Bulfon et al., 2013).

The use of plants and plant extracts has several positive aspects over the fish:

1. Growth and survival (SGR, FER, SR, WG, FBW)
2. Haematological profile (haematological indices and biochemical parameters)
3. Immune response
4. Resistance to infection (bacterial, parasitic, fungal and viral)

Moreover, research on mode of action, stability of plant materials in aquatic environment and digestibility in fish as well as *in vitro* and *in vivo* toxicological tests are prerequisites for their safe application (Bulfon et al., 2013).

Nowadays, only 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 than therapeutics, as the registration of herbal remedies to be used in this field is a time-consuming process and implies higher economic costs (Bulfon et al., 2013).

A2.4.3 Recommendations to the regulations - Knowledge gaps

- Anesthetic treatment shall not be included in allopathic treatment limitation.
- There is a conflict between the VMPs (all kind of Veterinary Medicine Products) current regulation and planned future VMP regulation and the organic regulation:
 - 1) The substances of preference (article 25t a-b-c) should be considered as feed raw material or additives.
 - 2) A more adequate procedure of authorization of relevant substances according to the new VMP regulation might be considered in relation to organic aquaculture
- Reconsider the setting of with-drawal period according to the VMP regulation, i.e. if a with-drawel period is not defined for a species or a product you can multiply by 1.5 the with-drawel period for a similar product registered for another species.

Knowledge gaps

- Herbal medicine should be further investigated as it may play a significant role as immunostimulant and as treatment tool in future organic aquaculture.

A3.0 Production systems

A3.1 Landbased culture – RAS - IMTA

A3.1.1 Introduction – Present regulations

According to Reg. 889/2008 6b: "Suitability of aquatic medium and sustainable management plan"

"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.

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" (EU, 2008).

According to Reg. 889/2008, Art. 25b: "Suitability of aquatic medium and sustainable management plan"; (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." (EU, 2008).

According to Reg. 889/2008, Art. 25c: "Simultaneous production of organic and non-organic aquaculture animals"; (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." (EU, 2008).

According to Reg. 889/2008 6d 1: «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» (IMTA) (EU, 2008).

According to Reg. 889/2008, Art. 25f: "General aquaculture husbandry rules";

"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.

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

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damage, other injuries, growth rate, behaviour expressed and overall health) and the water quality shall be monitored.

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.

4. Containment systems shall be designed, located and operated to minimize the risk of escape incidents.

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." (EU, 2008).

According to Reg. 889/2008 art. 25g on Specific rules for aquatic containment systems: "(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.

(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 inflowing and out-flowing water; (b) at least five percent of the perimeter ("land-water interface") area shall have natural vegetation.

(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.

"5. Ultraviolet light and ozone may be used only in hatcheries and nurseries", cf. EU, 2009, Art. 25s (5).

According to EU, 2008 Reg. 889/2008, Art. 25h: "Management of aquaculture animals"

"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.

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."

According to EU, 2008 Reg. 889/2008, Annex VII 2.1/2.2: "Products for cleaning and disinfection".

"2. Products for cleaning and disinfection for aquaculture animals and seaweed production referred to in Articles 6e(2), 25s(2) and 29a.

2.1. Substances for cleaning and disinfection of equipment and facilities, in the absence of aquaculture animals: — ozone — sodium chloride — sodium hypochlorite — calcium hypochlorite — lime (CaO, calcium oxide) — caustic soda — alcohol — hydrogen peroxide — organic acids (acetic acid, lactic acid, citric acid) — humic acid — peroxyacetic acids — iodophores — copper sulphate: only until 31 December 2015 — potassium permanganate — peracetic and peroctanoic acids — tea seed cake made of natural camelia seed (use restricted to shrimp production)

2.2. Limited list of substances for use in the presence of aquaculture animals: — limestone (calcium carbonate) for pH control — dolomite for pH correction (use restricted to shrimp production)

EXEPTION in Denmark regarding use of substances in the presence of animals

Currently, section 2.2 of Annex VII only includes limestone and dolomite as allowed for use in presence of aquatic animals. However, the possibility of using only these two substances is an urgent challenge for sustainable performance of the organic farming. Negotiations with the Danish authorities resulted in an amending specific authorization of the following substances to be allowed for use in presence of aquaculture animals in Danish organic production:

- rock salt/ sea salt
- hydrogen peroxide
- sodium percarbonate
- mixture of hydrogen peroxide and peracetic acid
- calcium hydroxide (slaked lime); used prior to inlet to the ponds/tanks.

According to Reg. 834/2007 art. 5 (c): In addition to the overall principles of organic farming focus shall be on the «recycling of wastes and by-products of plant and animal origin as input in plant and livestock production» (EU, 2007).

According to Reg. 834/2007, art. 15 1(b)(ii)(iii):» (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” (EU, 2007).

According to Reg. 889/2008, Annex XIIIa sec. 6: «Organic production of fish in inland waters: Species concerned: Carp family (Cyprinidae) and other associated species in the context of polyculture, including perch, pike, catfish, coregonids, sturgeon.»

”In fishponds ...periodically be fully drained and in lakes. Lakes must be devoted exclusively to organic production, including the growing of crops on dry areas. clean water inletoptimal comfort for the fish. The fish must be stored in clean water after harvest. Organic and mineral fertilisation of the ponds and lakes shall be carried out in compliance with Annex I to Regulation (EC) No 889/2008 with a maximum application of 20 kg Nitrogen/ha. Treatments involving synthetic chemicals for the control of hydrophytes and plant coverage present in production waters are prohibited. Areas of natural vegetation shall be maintained around inland water units as a buffer zone for external land areas not involved in the farming operation in accordance with the rules of organic aquaculture. For grow-out ‘polyculture’ shall be used on condition that the criteria laid down in the present specifications for the other species of lakes fish are duly adhered to” (IMTA) (EU, 2008)

A3.1.2 State of the art

Landbased aquaculture includes:

- Traditional flow-through pond farms
- Freshwater farming using recirculation technologies
- Land-based saltwater farms using recirculation technologies

Traditional farms use flow-through systems, in which the water is taken in via a damming of the adjacent water course/river and 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

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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.

Originally, fish production in fresh water (salmonids and others) occurred without any wastewater treatment. Thus, the production water was led directly to the water courses or lakes in which the fish farms were placed. However, during the last decades many fish farms have undergone gradual but significant technological development in terms of reducing the environmental impact from fish production.

One problem inherent to traditional farms was that damming of water courses by concrete constructions (weirs) hindered fauna mobility along the water courses when no efficient fish ladder or bypass is constructed. In particular, this problem concerns anadromous fish species, which may be hindered in reaching their spawning sites and the sea. To facilitate their migration along the water courses, the intake of fresh water from the water courses has been reduced via implementation of various technologies (e.g., re-use of water or recirculation technology); many dams and weirs have been removed; major bypasses have been constructed. Furthermore, grids can be installed at both the inlet and the outlet of the farms to prevent intrusion of wild fauna and escapement of fish from the farms (Jokumsen & Svendsen, 2010).

However, strict environmental regulations have been implemented in the aquaculture sector. The regulations includes feed utilization, in terms of the feed quotient expressed as the ratio kg feed fed/kg fish weight gain, that must be below a certain value; e.g. 1.0 for Danish portion sized trout (Jokumsen & Svendsen, 2010).

Furthermore, various water treatment units are required for mechanical (micro sieves, settling ponds) and biological filtration of the production water. Farms are also requested to consider Best Available Technology (BAT), e.g., farm construction and operating equipment, including cleaning devices, limitation of water consumption from the water course, feed composition and feeding management, process technology, oxygenation, vaccination, and use of medicine and chemical additives.

Due to stricter regulation, improved management, improved feed and feeding strategies the amount of fish produced per kg of feed has improved significantly, and the relative discharge of nutrients and organic matter from the fish farms has been lowered (Jokumsen & Svendsen, 2010).

As a consequence e.g. traditional trout farms have become more technological; i.e. they use varying degrees of water cleaning treatment, re-use of water, aeration, and oxygenation to meet the requirements. No standardized techniques have been applied, as fish farmers often use locally developed solutions.

However, due to unclear framework conditions for e.g. the trout farming sector in Denmark, there was a need of documentation of a relationship between fish farming and its direct impacts on the adjacent aquatic environment. Furthermore, there has been no documentation of the environmental effects of the different technologies applied on the fish farms. This broadly recognized fact resulted in the idea of the Danish model trout farms.

Model Freshwater Fish Farm

The aims of the model fish farms were to:

- Provide documentation about the management of and environmental parameters relevant to the fish farms, including documentation of the specific discharge of
 - Nitrogen (ammonia, nitrate, and total nitrogen)
 - Phosphorus (dissolved and total)
 - Biochemical Oxygen Demand (BOD), which expresses the rate of oxygen uptake by micro-organisms in a sample of water at a temperature of 20 °C in the dark
 - Chemical Oxygen Demand (COD), which is a measure of the content of organic matter in water
 - Documentation and determination of the efficiencies of specific cleaning devices (micro sieves, sludge cones, biofilters, and plant lagoons)
- Reduce the intake of fresh water
- Increase the retention/transformation of organic matter and of nutrients
- Meet the environmental quality goals for the specific recipient (river, lakes, coastal areas)
- Increase the fish production without a corresponding negative environmental impact
- Facilitate administrative procedures to streamline and ease environmental approvals.

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 & Svendsen, 2010).

Model farm type 1

Model farms of this type are extensive farms with mechanical water treatment and reuse of water (maximum 1.25 l water/sec/ton feed/year). 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 & Svendsen, 2010).

Model farm type 2

Model trout farms of this type are intensive farms with mechanical and biological water treatment and 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.

Model farm type 3

The model farms type 3 represent the highest level of innovation with the lowest consumption of new water. The maximum value is 0.15 l water/sec/ton feed/year or 3,600 l 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 about a factor of 15–25 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., a bore hole (well), 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.

Environmental benefits of Model Farms

The reduced and stabile water intake that characterizes the model trout farms is beneficial to the environment, but it has both advantages and disadvantages for the management of the farm, cf. the following table:

Water course	Fish farm
<p>Advantages:</p> <ul style="list-style-type: none"> • Free water flow up/down stream and natural variations in the water flow of the water course facilitated by water bypass • No or reduced effect of damming • Free fauna passage • Reduced nutrient and organic matter losses per kg produced fish • Reduced discharge of medicines and therapeutants and reduced maximum concentrations • Improved oxygen conditions downstream of the fish farm • Reduced losses of fauna from water courses to the fish farm <p>Disadvantages:</p> <ul style="list-style-type: none"> • None 	<p>Advantages:</p> <ul style="list-style-type: none"> • Stable production conditions • Minor variations in water quality • Improved efficiency of cleaning devices • Using water from bore hole leads to fewer seasonal temperature variations • Improved control of management and production • Reduced external risk of infection with pathogens • Reduced need for medicine and therapeutants • Improved work environment <p>Disadvantages:</p> <ul style="list-style-type: none"> • Higher energy consumption per kg fish • Increased discharge of CO₂ • Risk of toxic levels of ammonia and risk of disagreeable taste in fish meat • Increased need of supervision and management • Increased need of back-up systems: Electricity, oxygen, pumps, etc.

The investigations of the model farms have shown significant reduced losses of nutrients and organic matter to rivers and streams associated with the production compared to the discharges from traditional fish farms.

Organic Fish Farms

Landbased organic fish farms follows a more classical design being extensive compared to e.g. model 3 farms. According to EC 710/2009, Art. 25g (EU, 2009) intensive recirculation technologies are only permitted in hatcheries and nurseries, while extensive recirculation of water is permitted for on-growing. No upper limits are given in the regulation, but in practice up to about 70 % recirculation for on-growing is approved by Danish authorities (Korsholm, 2014; pers. Comm.).

In addition to the organic regulation (EU, 2009) organic farms shall as well fulfill national regulations for conventional farming.

IMTA (Integrated MultiTrophic Aquaculture)

Integrated Multitrophic Aquaculture (IMTA) or Aquaponics is defined as a sustainable system combining traditional aquaculture with hydroponics, where plants are cultivated in water. Effluents from the aquaculture are used as nutrients for the plants in the hydroponics, thus creating a symbiotic natural environment with maximum use of all raw materials and waste. The water from the plants is then recycled back to the fish tanks, making the IMTA a potential productive food systems in terms of water, energy and nutrient efficiency.

However, the main challenge for is to maximize the overall economic output by synchronizing the various biological growth periods and optimums of fish, plants and microorganisms into a stable and balanced biomass production system. Optimum solutions to tradeoffs between the best environmental parameters for different species have to be developed. At the same time high skills are required when operating on a larger scale commercial basis, balancing between the need for securing a stable biological system and being flexible towards a market demand with fluctuating prices and changing consumer preferences (Rakocy, 2010, Martins et al., 2009, Timmons and Ebelings, 2010).

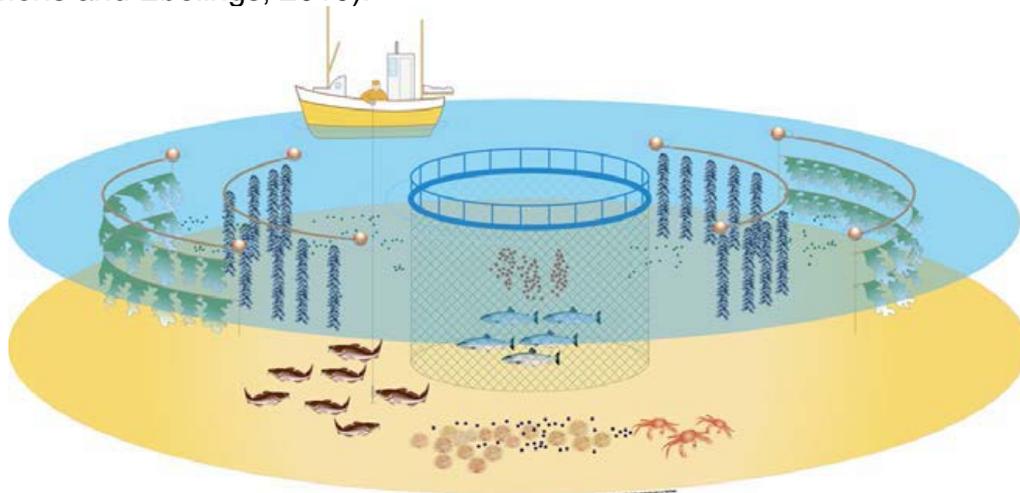


Illustration of Integrated Multi-Trophic Aquaculture (IMTA).

A3.1.3 Recommendations to the regulations - Knowledge gaps

Based on interpretation of the current regulation EC Reg. 710/2009, (EU, 2009) the Danish authorities on inspection of organic aquaculture facilities has prepared a kind of check-lists for approving and control of organic production facilities (Larsen, 2014).

The list regarding land Based Culture included (Larsen, 2014):

Annex XIIIa, Section 1:

Salmonids in freshwater (FW): Open systems – do not exclude recirculation.

- Min. 60 % oxygen saturation
- Salmon, arctic char: max. 20 kg/m³
- Sea- and rainbow trout: 25 kg/m³

Annex XIIIa, Section 3:

Cod, bass, bream, turbot, ..in sea and on land: Production in cages at sea or in open land-based facilities with sufficient water current for optimum welfare.

- Turbot: 25 kg/m²
- Others: 15 kg/m³

Annex XIIIa, Section 4:

Bass, grey mullet, eel in earth ponds in tidal zones or coastal lagoons: Secure adequate renewal of water for welfare. 50 % of dikes must have plant cover. Wetland based depuration ponds required.

- Max. 4 kg/m³

Annex XIIIa, Section 5:

Sturgeon in fresh water: Secure adequate stream for welfare. Discharged water shall have same quality as intake water.

- Max. 30 kg/m³

Annex XIIIa, Section 6 and 25f 1e:

Carp family fish and other sp. in polyculture (perch, pike, catfish, coregonoids and sturgeon). Ponds which regularly are emptied completely and in lakes. Organic rules must include the whole lake and crops in adjoining areas. Sufficient renewal of water to secure fish welfare. Organic and mineral nutrients may be added, though max. 20 kg N pr. ha. Use of synthetic chemicals against water plants and plant cover is not permitted. Buffer zone with natural vegetation in areas not belonging to the farm. In polycultures the criteria must be fulfilled for all fish in the farm.

Carp must have access to bottom of natural earth.

Annex XIIIa, Section 9:

Milkfish, tilapia and siamese catfish: Ponds and cages

- Tilapia - Max. 20 kg/m³
- Siamese catfish - Max. 10 kg/m³

Ch. 1a, art. 6b,1: Siting

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.

Ch. 1a, art. 6b, 2: Separation Organic ctr. Conventional

2. Organic and non-organic production units shall be separated adequately (In Denmark 500 m according to Inspection authority). 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.

Ch. 1a, art. 6b, 3: Environmental assessment for production units > 20 T prod./y.
Responsibility of the operator.

Ch. 1a, art. 6b, 4-5 and 25b 2: Plan for sustainable management

To be provided by the operator. Environmental impact (N & P). Maintenance of equipment/regular test; energy sources (preferably renewable energy); strategy for reduction of waste, re-use of materials, Habitat directive, national regulations; Log-book.

Art. 25b 4: Farming systems

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.

Art. 25c 1: Simultaneous production

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.

Art. 25d 1 & 2: Origin of organic aquaculture animals

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.

2. Species shall be chosen which can be farmed without causing significant damage to wild stocks.

Art. 25e 1: Origin of non-organic fish

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

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holding. Such animals shall be kept under organic management for at least three months before they may be used for breeding.

Art. 25e 2: Recruitment of fry

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.

Art. 25e 3: Use of conventional fry

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. DK interpretation for conversion period: For FW fry max. 50 g. For SW conventional fish weighing X gram on arrival can only be sold as organic at 3*X gram.

Art. 25e 4: Wild fry

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.

Art. 25f 1: Farming environment

- (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; i.e. (DK): Concrete bottom OK if sandhives/areas with sand/gravel (> 25 % of bottom area) available – only outdoor facilities – not hatcheries. Otter net if needed to minimize stress.

Art. 25f 2: Stocking density

Cf. Annex XIIIa and above. DK interpretation: Density limits also apply for hatcheries/ fingerling units.

Art. 25f 3: Farming water

Oxygen: min. 60 %. To be measured daily at the fish, and in inlet (not outlet). All units measured once/week. Inlet each day.

pH (trout): 6-8

Nitrate: Max. 300 mg/l

Nitrite: Max. 5 mg/l

Ammonium: Max. 5 mg/l

Ammonia: Max. 0.1 mg/l

Apply for traditional farms and farms using recirculation below 50 %, to adjust the measuring intensity to the intensity of farming (recirculation).

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Art. 25f 4-5: Escape

Containment systems shall be designed, located and operated to minimize the risk of escape incidents.

Inlet and outlet must be approved by the authorities.

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.

Art. 25g: Containments systems

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.

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 percent of the perimeter (“land-water interface”) area shall have natural vegetation.(NEED interpretation!).

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.

Emergency generator should be available.

All such use is to be recorded in the aquaculture production record.

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.

Knowledge gaps

”Recent technical development has led to increasing use of closed recirculation systems for aquaculture production. However, such systems depend on external input and high energy but permit reduction of waste discharges and prevention of escapes. Due to the principle that organic production should be as close as possible to nature the use of such systems has not so far been allowed for organic production. Exceptional use has only been possible for hatcheries and nurseries”, cf. EU, 2009, rec. 11. However, further knowledge is needed about RAS and IMTA and the potential use of the technologies in organic aquaculture. This also should be communicated to the consumers, who are hesitant to advanced technologies in organic aquaculture – and generally in the perspective of organic principles close to nature (soil and water).

A3.2 Breeding - Hatchery

A3.2.1 Introduction: Present regulations

According to Reg. 834/2007; Art. 15 1(c) re: Production rules for aquaculture animals: "with regard to breeding: (1) artificial induction of polyploidy, artificial hybridisation, cloning and production of monosex strains, except by hand sorting, shall not be used; (2) the appropriate strains shall be chosen; (3) species-specific conditions for broodstock management, breeding and juvenile production shall be established;" (EU, 2007).

According to EC Reg. 889/2008, art. 25d re.: Origin of organic aquaculture animals: "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. 2.) Species shall be chosen which can be farmed without causing significant damage to wild stocks" (EU, 2008).

According to Reg. 889/2008, art. 25e 1 re.: Origin and management of non-organic aquaculture animals. "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." (EU, 2008).

According to Reg. 889/2008, art. 25e 3 re.: Origin and management of non-organic aquaculture animals. "The maximum percentage of non-organic aquaculture juveniles introduced to the farm shall be: 80 % by 31 December 2011, 50 % by 31 December 2013 and 0 % by 31 December 2015." (EU, 2008).

According to EC Reg. 889/2008, art. 25h 2 re: Management of aquaculture animals. "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." (EU, 2008).

According to Reg. 889/2008, art. 25i re: Prohibition of hormones. "The use of hormones and hormone derivatives is prohibited." (EU, 2008).

Hatchery

According to Reg. 834/2007; Art. 15 1(a) re: Production rules for aquaculture animals. "with regard to the origin of the aquaculture animals: (1) organic aquaculture shall be based on the rearing of young stock originating from organic broodstock and organic holdings; (2) when young stock from organic broodstock or holdings are not available, non-organically produced animals may be brought onto a holding under specific conditions;" (EU, 2007).

According to Reg. 889/2008, art. 25c 1 re: Simultaneous production of organic and non-organic aquaculture animals. "The competent authority may permit hatcheries and nurseries to rear both organic and non-organic juveniles in the same holding provided there is clear

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physical separation between the units and a separate water distribution system exists.” (EU, 2008).

According to Reg. 889/2008, art. 25e 4 re: Origin and management of non-organic aquaculture animals. ” 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.” (EU, 2008).

According to Reg. 889/2008, art. 25g 1,4 re: Specific rules for aquatic containment systems. ” 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.” 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.” (EU, 2008).

Carp (and other species in carp ponds) reproduction is often managed naturally and not in hatcheries. It is clearly stated that reproduction induced by hormones and hormone derivatives, as well as artificial induction of polyploidy, artificial hybridisation, cloning and production of monosex strains are all practices incompatible with organic production. However, also the opinion, that pituitary hormones could be acceptable as they have a short half-life, has already appeared (Rodriquez Nunez 2005). The stock should come from organic broodstock and from organic farms, but until 2015, non-organic fish can be introduced under certain conditions and labelled organic as long as at least the latter two-thirds of their life have been spent under organic management.

According to Reg. 889/2008, art. 25o 1(b): Re Sourcing of seed 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” (EU, 2008).

According to Reg. 889/2008, art. 25s 5: ”Ultraviolet light and ozone may be used only in hatcheries and nurseries” (EU, 2008).

According to Reg. 889/2008, Annex VII 2.1/2.2: ”Products for cleaning and disinfection” cf. samme text in section 5.1.1. (EU, 2008).

According to Reg. 889/2008 art. 25a: ”..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, cf. Annex I, V and VI (EU, 2008).

A3.2.2 State of the art

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. Further, breeding shall aim at strains which are well adapted to farming conditions, good health and high feed utilisation. Organic fry shall be robust to diseases due to the limitations of allopathic and chemical treatments.

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; TROUTEX, 2014). Therefore, the present production of organic juveniles may be inadequate to supply the growing demand of the organic aquaculture industry.

In order to facilitate the fulfilment of the organic rules on organic juveniles an option to secure supply of organic breeding material and juveniles without running a separate breeding population, might be to use the sibs of the breeding candidates from a conventional breeding program by choosing families with the best breeding values for certain traits such as disease and/or parasite resistance. Breeding companies routinely challenge sibs of the future selection candidates with different diseases in controlled challenge tests. These tests are usually performed shortly after tagging size, which means that information of disease resistance of each family is available at an early stage. Sibs within those families identified as highly resistant can then be selected based on their growth performance, reared according to organic standards for a minimum of 3 months and then used to produce organic juveniles. In order to access if breeding objectives between conventional and organic farmers differ, economic values for each traits in the breeding objective needs to be calculated.

It is important to avoid inbreeding in order to supply juveniles selected for good performance in terms of growth, resistance to disease and survival. Inbreeding occurs when related animals are mated, and may lead to inbreeding depression compromising performance. Inbreeding is as such inevitable in a closed breeding population, because at a certain moment it is impossible to avoid mating of unrelated individuals completely. In mass selection programs it has been shown (Bentsen and Olesen, 2002) that a minimum of 50 pair of breeders should be selected to keep rate of inbreeding at 1 %, which is a generally accepted level to avoid loss of fitness in a given breeding program (Meuwissen and Woolliams, 1994; Pante et al., 2001).

Even the regulation opens for using wild caught fish for organic production it shall be noticed that wild caught animals have not gone through a domestication process and are not adapted to modern aquaculture production. This may impact the welfare of the fish in terms of more stress, aggression, cannibalism, diseases and mortality. In addition, domesticated stocks have to a high degree been selected for increased growth and thus have a higher productivity with accompanied improved feed efficiency than wild caught fish. In addition, capturing animals from the wild may have a negative effect on the wild populations. Thus the breeding stock should be based on a domesticated population

However, it is challenging for breeding companies to develop a genetic material, which is specifically developed for the organic farmers. It requires a lot of resources and there should be a high demand for organic roe for the breeding companies to justify the costs of a separate

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breeding program. Hence, except for trout and maybe salmon, it might be challenging to fulfil the regulations' requested maximum of non-organic aquaculture juveniles introduced to the farm of 50 % by 31st December 2014 and 0 % by 31st December 2015.

Besides the lack of organic juveniles, due to the few hatcheries certified as organic, attention should also be paid to 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.).

Furthermore, the current regulation does not provide any 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, husbandry environment) and, even more, marine species (e.g. phytoplankton and zooplankton production, essential nutrients in the trophic chain, stocking density during larval rearing and weaning, husbandry environment). Hence, it may be difficult to distinguish organic and non-organic hatcheries.

However, 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; TROUTEX, 2014).

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.

Marine hatcheries - Phyto- and Zooplankton (live larval feed)

Larval rearing is one of the most critical stages for the successful start of life of any species and represents one of the major bottlenecks of the whole aquaculture production. 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 the rearing environment and to the feed quality. These small larvae are not able to ingest artificial dry feed but need live feed as first feeding, i.e. live zooplankton (e.g. small crustaceans), which fulfil the specific requirements to nutritional composition and size. Therefore the hatcheries need to include facilities for plankton production (both phytoplankton and zooplankton), tanks for larval rearing and as well as for weaning to dry feed (few weeks after first feeding on live feed). Facilities for brood stock are also needed, although eggs may be purchased from other hatcheries to initiate a production cycle.

Phytoplankton

Phytoplankton is of major importance in the hatchery process, having a double role. It is used in the zooplankton cultures (e.g. rotifers) 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 but also shading effect that improves larvae predation or as trigger for feeding behavior or physiological processes (Scott & Baynes 1979; Naas et al. 1992; Tamaru et al. 1993; Reitan et al. 1993; Cahu et al. 1998; Van der Meer 1991).

The mass culture of phytoplankton is usually performed in plastic bags or in photobioreactors at high cell density (Tredici & Materassi 1992; Pulz 2001).

Nutrients are needed to be added for mass-production of phytoplankton. Commercial nutrient solutions contain all necessary macro- and micronutrients, silicates and vitamins in easily soluble, mineral form (Vonshak, 1986; Smith et al., 1993; Lavens & Sorgeloos, 1996).

However, 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.

Accordingly, it is currently not possible to define production of "organic phytoplankton" which would be sufficiently different from conventional phytoplankton.

Zooplankton

For mass-production of zooplankton as live feed for larvae of marine fish two species of zooplankton are mass cultured due to their appropriate size and easiness of mass culture. These are (1) the rotifer *Brachionus* sp. and (2) 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 & Sorgeloos, 1996, Marcus 2005).

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).

Rotifers and *Artemia* need to 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 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.

Unlike phytoplankton, it might be possible to produce organic zooplankton. This would require use of organic yeast and other microorganisms, natural antioxidants and emulsifiers.

Larvae, post-larvae and juveniles

A variety of hatchery techniques are available (Divanach & Kentouri, 1999), all sharing a common characteristic i.e. the use of plankton (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: (1) egg hatching and autotrophic phase when larvae consume their yolk sac reserves, (2) heterotrophic phase when larvae are fed on zooplankton, and (3) 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. This general scheme applies for both marine and freshwater 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.

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 & Kentouri 1999), have been developed and are applied for the rearing of several species. The most important characteristic of the infrastructure required is the size of the larval tanks which should range between 20 to 60 m³. The initial egg density in the mesocosm ranges from 4 to 7 eggs/l, depending on species, and should never exceed 20 eggs/l. 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 *Artemia* sp. and artificial diet is added when required (Papandroulakis et al. 2004; Kentouri & 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. 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 seabream 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 behavioral response of sea bass during on-growing, and the individuals reared with the mesocosm method are more sensitive to human presence, presenting behavior closer to wild individuals (Papandroulakis et al., 2012).

Larval rearing of fresh water species (percid)

The larval rearing of pike-perch 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, 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., 2007) or, seldom, to non-enriched *Artemia nauplii*. This change occurs gradually, largely depending on the size of the fish mouth, that 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 (Adamek, 2014, pers. comm).

Larval rearing of molluscs and crustaceans

Mollusc larvae and start-feeding shrimp larvae are filter feeders and consequently feed on phytoplankton. Later stages of shrimp and most other crustacean larvae are first fed on *Artemia* and later on microdiets.

For mollusc rearing, low densities are commonly used. For crustaceans, stocking density has variable effects: shrimp postlarvae can be kept at higher densities, while this is not the case for prawns and even less for crab and lobster.

For marine fish, it seems that juveniles produced with "mesocosm" or "large volume rearing" systems are more similar in behaviour and morphology than their wild counterparts. With respect to freshwater fish, molluscs and crustaceans, there is a knowledge gap.

A3.2.3. Recommendations to the regulations - Knowledge gaps

- 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 incl. phyto- and zooplankton and larval rearing systems
- If available, domesticated and unrelated broodstock, preferably selected for relevant robust traits (survival, disease resistance and growth) should be used in breeding for organic seed

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- Need of defining breeding objectives and implement cost effective breeding strategies that control inbreeding rate at a sufficient low level (<0.5% per generation) to secure adequate genetic material specifically for organic aquaculture

A4.0 Potential Environmental Impacts and Interactions

A4.1 Escapee

A4.1.1 Introduction: Present regulation

According to Reg. 889/2008, art. 25f 4/5: «4. Containment systems shall be designed, located and operated to minimize the risk of escape incidents. 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» (EU, 2008).

A4.1.2 State of the art

Escapes are a problem for almost all European farmed finfish species. 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 legislative and reporting requirements in Europe (Jackson et al., 2013).

Escapes from cage aquaculture is normally related to escapes of 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 (Jørstad et al. 2008) has also shown to be an escape risk (Dimitriou et al., 2007; Somarakis et al., 2013). Hence, escapes should be distinguished between 1) escaping individuals and 2) escapes via spawning.

The main causes of escapes in Norwegian cages are due to structural factors (Jensen et al., 2010). Escapes can 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.

However, a marked decrease in the number of escapes from Norwegian net cages has happened in recent years due to national legislation to make efforts (physical design, installation and management) to reduce escapes from commercial Norwegian cage salmon farming (Jensen et al., 2010) in line with art. 25f, 4 in Reg. 889/2008 (EU, 2008, cf. 6.1.1)

However, other cage farmed species such as Atlantic cod and gilthead seabream may 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., 2008; Glaropoulos et al., 2012). But rather simple cost effective management procedures may also reduce these risk of escapes, i.e. using better and more robust netting materials, matching the colour of net repairs to the existing net (Damsgård et al., 2012) or cage enrichment (Zimmermann et al., 2012).

Escapee of farmed fish is an unintended event as it both is a loss of money for the farmer and may impact wild fish stocks. However, in case of an escapee event, precautions should be taken, e.g. recapturing of the escaped fish. Fish may disperse rapidly and widely after an escape event and can disperse >1km from the farm in a few hours (Whoriskey et al., 2006). However, rapid migration is not always the case as 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).

Investigations on recapture of escaped Atlantic salmon, cod and seabream showed great variations using bagnet, gillnet, acoustic signals, angling and local fishermen but the majority of the released fish were recaptured (Chittenden et al., 2011; Hansen and Youngson, 2010; Skilbrei et al., 2010; Tlusty et al., 2008; Uglem et al., 2008; Arechavala-Lopez et al., 2012).

A4.1.3. Recommendations to the regulations - Knowledge gaps

- Species-specific distinctions might be made between escapes of fish and escapes of viable gametes within the EU Organic regulations
- Put efforts on prevention of escapees, i.e. putting requirements for the physical design of the installation of net cages, i.e. calculation and design, operating and maintenance requirements
- Put specifications on robust netting materials to resist tearing or biting
- Use of a curtain-like egg collector to mitigate against egg escapee in cages with potential spawners (Atlantic cod and gilthead seabream)

Knowledge gaps:

- Test of commercial efficacy of curtain-like egg collectors to mitigate against occurrence of egg escape
- Investigating the efficacy of using environmental enrichment to reduce net biting frequency
- Improvements of recapture methods

A4.2 Energy consumption – CO₂ emission – Life Cycle Analysis (LCA)

A4.2.1 Introduction: Present regulations

According to EU 889/2008 art. 6b 5 the present regulations states that “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” (EU, 2008).

EU 889/2008 art. 25h 3 states, that “Aeration is permitted to ensure animal welfare and health, under the condition that mechanical aerators are preferably powered by renewable energy sources”. In art. 2 k energy from renewable sources is defined as renewable non-fossil energy sources: wind, solar geothermal, wave, hydropower, landfill gas, sewage treatment plant and biogases (EU, 2008).

There are no specific regulations on the release of CO₂ (carbon footprint) and global warming potential (GWP), which however is closely related to the use of fossil energy.

A4.2.2 State of the art

Energy is used in all parts of aquaculture production, from farming system, 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

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fishing vessels, farm machinery and for transport processes throughout the value chain. Currently power from renewable sources like hydropower and windpower are mixed with non-renewable energy from coal and natural gas. To be sure of using renewable energy an aquaculture facility would need to have its own power supply from wind, sun or biogas. However, most of the total energy consumed during the production process is used when growing, harvesting, processing and transporting the feed ingredients (Ellingsen and Aanonsen, 2006, Tyedmers et al., 2007, Ellingsen et al., 2009, Pelletier et al., 2009, Boissy et al., 2011, Skontorp et al., 2011). Hence, the regulations laid down for feed composition in organic production will be a direct driver for how much energy that will be used in the production. The general rules on feed for fish, crustaceans and echinoderms states that feeding regimes should be designed to create low environmental impact (EU 889/2008 art. 25 j) and the rationale behind organic food production is to produce food with the lowest possible environmental impact, though also considering other aspects such as animal welfare, product quality and social aspects - farmer welfare (profit, health). Skontorp et al., 2011 estimated that the dietary marine and plant ingredients accounted for about 90 % of the CO₂ emission and energy consumption in salmon production.

Energy consumption and global warming potential in aquaculture - LCA studies

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 2011b). 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 need to be produced globally within 2050 to feed a population of 9 billion people and calls for urgent action in developing food systems that uses less energy and emits less greenhouse gases (FAO 2011c). At present there are few standards or reference/threshold values for what can be defined as sustainable food production. But in recent years there has been increasing interest for developing models, metrics and tools 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.

Life Cycle Assessment (LCA) has in the last decade become the most standardised method for assessing environmental performance. It 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, including input of energy and resources associated with each stage from resource extraction and processing, consumption, disposal and recycling.

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 fish production

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(Ellingsen and Aanonsen, 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 CO₂ released when producing the feed ingredients is very important for the cumulative energy consumption and global warming potential of aquaculture production. The regulation for organic aquaculture states 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 (currently until 31. December 2014) also 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 Aanonsen (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 by 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 CO₂ 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 CO₂ 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 fish diets, both in conventional and organic aquaculture.

Plant ingredients are very variable with respect to environmental impacts. A comparison of rapeseed oil and palm oil showed 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 currently approved in organic aquaculture feeds. Organic production of the main ingredients used in conventional diets (canola, soy, wheat and corn) was estimated to consume 60 % less energy and reduce the CO₂ 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.

Due to that the majority of the energy and global warming potential of fish production is associated with the feed production, efficient use of this feed for aquaculture 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 aquaculture. Fish meal from trimmings and offal contain less 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 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 the production will also increase by around 10 % because 90 % of the CO₂ emissions in fish farming are related to feed production and transport of feed.

A LCA comparing certified organic to conventional fish feed (Pelletier and Tyedmers, 2007) showed that organic crop ingredients had a lower life cycle impact compared to conventional crops, but this effect was outnumbered by larger impact of fish- or animal derived ingredients.

A4.2.3 Recommendations to the regulations - Knowledge gaps

- Lack of defined criteria and reference points for an environmental sustainable food production

A4.3 Recycling and waste

A4.3.1 Introduction: Present regulations

According to EU Reg. 834/2007 art. 5 c: In line with the overall principles organic farming should include: “the recycling of wastes and by-products of plant and animal origin as input in plant and livestock production” (EU, 2007).

According to EU Reg. 889/2008, art. 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 (EU, 2008).

According to EU Reg. 889/2008, art. 6d, 4: “Ropes and other equipment used for growing seaweed shall be re- used or recycled where possible” (EU, 2008).

A4.3.2 State of the art

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. Extruded fish feed has high nutrient density, and feed spill and undigested material results in loss of significant amounts of nutrients and energy. Whereas fish feed earlier was mainly based on fish meal and fish oil, today’s feed contains an increasing amount of plant ingredients (Ytrestøyl et al., 2011), which results in an increasing amount of indigestible fiber and thus waste. The apparent digestibility of the dry matter may typically be approximately 65 % in high quality fish feeds of commercial type for non-organic farming (Oehme et al., 2013). Hence, 35 % of the dry matter of the feed will be lost as undigested material, which, with a FCR of 1.3, corresponds to approximately 450 kg of dry matter from faeces per ton of fish produced.

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

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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 fish 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, the dry matter content in sludge is highly variable and 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 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 few 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 prevent decomposition and hygienizes the sludge (Bergheim et al., 1998).

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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 phase. 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. 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 fish 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.

A4.3.3 Recommendation to the regulations - Knowledge gaps

- Need of knowledge and technology for recycling of nutrients from aquaculture
- Investigations of solutions for collection, de-watering and re-use of waste from aquaculture production

A4.4 Sea bottom

A4.4.1 Introduction: Present regulations

According to EU Reg. 889/2008, art. 25q 2 on molluscs: "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", EU, 2008).

According to EU Reg. 889/2008, art. 25g 3 on aquatic containment systems at sea shall: « 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», EU, 2008.

A4.4.2 State of the art

The EC regulations regarding environmental impact and interactions in relation to the sea bottom are not very specific. However, the Norwegian organic label Debio which regulates organic production in Norway is based on this EC regulation, but put specific requirements that no significant organic sediments should build up under the farms. The organic environmental load (feed waste and faecal material) should be minimised to avoid eutrophication. Each location has to undertake a recipient inspection to ensure no negative environmental impact.

Cage farms may also 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.

Only little knowledge is available regarding the amount of lost feed in sea cages 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 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 diet. 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.

Wild fish feeding on feed spill from cages

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 & 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 (Sanchez-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 et 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 cage farms within which wild fish are attracted is not known. In the Aegean 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 Aegean Sea not only aggregate fish, but also leads to increased wild fish production possibly due to increased nutrient availability (Machias et al. 2005; 2006).

FAD and wild fish feeding on waste feed may have additional ecological impacts. 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., 2003; Dempster et al., 2009; Otterå et al., 2009; Bustnes et al., 2010; Arechavala et al., 2010;2014; 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 al., 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 (Sargent et al., 2002). Some essential fatty

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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 between them 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).

A4.4.3 Recommendation to the regulations - Knowledge gaps

- Environmental impact and interactions in relation to cage farming and the sea bottom needs consideration
- Ecological impact of cage farming and wild fish attracting device needs consideration

A5.0 Consumer – Socio-economy – Institutional Frameworks

In WP 3 a review on available information on economic, market and consumer related issues as well as regulatory and institutional frameworks related to organic aquaculture are being performed. The review is an ongoing work which will be presented in the M18 deliverable, including a consumer survey based on the gaps revealed in literature review and the stakeholder feedback from the first stakeholder meeting.

This section gives a brief summary of the preliminary findings.

A5.1 Literature review on consumer perspectives

- The image of aquaculture is transferred to the image of fish from organic aquaculture. Consumers that are positive about aquaculture are also positive about organic aquaculture and if they are negative, they remain negative. ([Verbeke et al., 2007; Davidson et al., 2012; Polymeros et al., 2014])
- Consumers that are positive about organic production are also willing to pay for organic. This link is however stronger when consumers have high education, high income, high knowledge about organic and if they have young children. (Kesse-Guyot et al., 2013; Dagistan et al., 2009)
- Additional issues that positively influence the acceptance and use of organic fish are the health benefits of such fish, the naturalness of the production, that the production is local or at least domestic and the food safety associated with such a production method. (Stefani, Scarpa & Cavicci, 2012; Miele, 2001)
- Very little knowledge exists about how consumers perceive types of feed used in aquaculture
- Quality variations might be perceived by consumers when fish are produced under good welfare regimes. Most consumers are not concerned about fish welfare, while a small segment is. (Sogn-Grundvåg, Larsen, & Young, 2012; Grimsrud et al., 2013; Kupsala, Jokinen & Vinnari, 2005; Frewer et al., 2005; Sveinsdottir et al., 2010)
- Very limited knowledge about consumers' perceptions of various production systems. Lack of knowledge about production systems may lead to shock from exposure to information about production details. (Kaiser & Stead, 2002)
- When consumers realise that aquaculture protects wild stocks, they perceive aquaculture as something good for the environment. Level of knowledge about aquaculture can lead to either emotional or logical development of the image consumers have about aquaculture. Emotional development tends to lead to a negative image, while a logic driven development leads to a more positive image of aquaculture and its global impact to the environment. (Teisl, Fein & Levy, 2009; Honkanen & Olsen, 2009; Vanhonacker et al., 2013)
- The concept of organic is not one common understanding among consumers. Biological, organic, ecological, green, sustainable and even fair trade labelling is closely related in the eyes of consumers. Distrust is influenced by knowledge about organic fish, perceived transparency of the controlling agents, tangibility of the provided information, perceived honesty of the carrier of the information and the source of information (e.g. Official/Government vs. Commercial/Industry). (Pieniak, Vanhonacker & Verbeke, 2013; Pieniak et al., 2007; Aarset et al., 2004)

- Increased familiarity with a label, perceived increased personal health benefits, societal benefits related to the environment, increased education levels, increased income, older age and additional interest in ecological issues lead consumers to increased willingness to pay for products that carry organic label. (Salladarre et al., 2010; Krystallis & Chryssohoidis, 2010; Hoogland, de Boer & Boersema, 2007; Xu et al., 2012, Solgaard & Yang, 2011)

A5.1.1. Knowledge gaps in consumer literature

The literature review revealed that there is a good knowledge base on the topics related to consumers and their perception of fish and aquaculture in general, organic food in general, organic aquaculture as a whole, fish welfare perception, environmental impact and sustainability of aquaculture, general label perception regarding environmental issues, specific perception of eco/organic/welfare/sustainability labelling and opinions about certification schemes and the way they are controlled. However, important knowledge gaps in consumer perceptions were also identified for two of the main topics related to organic aquaculture; specific production systems and feed used in production systems.

Very limited knowledge about consumers' perceptions of **production systems** exists. Production systems that are perceived as more natural are preferred by consumers that do not report a need for much information about production details, but would like to see higher transparency and public involvement in decisions related to aquaculture production (Feucht & Zander, 2014; Kaiser & Stead, 2002). Looking at grey literature provided some inspiration about relevant topics to study further during the development of the consumer survey. There was particular focus on the amount and type of information, as well as the sources of this information about production systems and the unpredictable effects they may have until they are tested (Kole, Altintzoglou, Schelvis-Smit, & Luten, 2009; Robertson & Carlsen, 2000). Therefore, the information about production systems to which the consumers will be exposed to need to be carefully prepared in order to represent a balanced, objective and comprehensible description of each system. The latter could be achieved with a higher abstraction level used in the description of the system, referring to resulting effects of the system on fish welfare and avoiding crude production details. Looking at organic food literature and the production systems related to other species made quickly obvious that the production system alternatives are not directly comparable to those of aquaculture. The existence of water treatment, multi-trophic opportunities and options between sea or land placement of the production have no parallel to the production of beef, pork, eggs or vegetables. We therefore limited the knowledge use to methodologies for evaluating consumer perceptions regarding ethical issues related to the results of production systems on the image of the system (e.g. naturalness, environment, etc.).

Almost no published research was found on the topic of **feed** ingredients for organic or conventional aquaculture and how consumers perceive the impact feed has on the health and welfare of the animals and therefore their own (Kole, Kremer, Honkanen, Mejdell, & Schelvis, 2008). There are conflicting results as to if consumers can perceive differences between fish products depending on the feed used (Jokumsen, 2008; Schacht & Busch-Stockfisch, 2009). However, the letter study did not report consumers' perception of the feeding system. Australian consumers generally preferred conventional fish meal feed for conventional aquaculture and a segment of the participants may also positive towards GM oilseed (Cox, Evans, & Lease, 2008). Looking at organic food in general, it has been suggested that the

specificity of feed type is a communication dimension that may attract different consumers than the concept of organic food as a total (Janssen, Heid, & Hamm, 2009). Therefore, referring to details of the organic concept may make consumers more responsive to the tangibility of the effects of their shopping choices. The effect of feed on the expected quality of a fish product is a topic interesting for further investigation in the consumer survey.

Knowledge gaps used in survey design

Results from the review of relevant literature led to conclusions that can be used as input for the adaptation of regulations regarding organic aquaculture. However, knowledge gaps were also identified and were covered by the design and performance of a consumer survey in four countries: UK, France, Italy and Germany. The survey design was based on established scientific methodology used on consumer research on organic food and based on relevant parts of the regulatory framework (EC 834/2007, 710/2009). The survey started with questions about terms and concepts consumers regarded relevant to the EU official definition of organic fish and how some of them can influence the quality of the fish (including feed, production system characteristics, etc.). Then the survey included questions about consumers' attitudes towards organic fish and their familiarity and use of relevant labels. Perceptions and attitudes (including naturalness, quality, health, sustainability, safety, etc.) about wild, conventional farmed and organic fish were also reported by consumers. Consumers also reported their knowledge about organic fish, their consumption behaviour and socio-demographic characteristics.

References

A5.2 Survey – preliminary results

- The objective (actual) knowledge concerning organic fish was just above medium in this sample, with an average of about 57% correct answers given with an average certainty of 3 out of 5. On the contrary, self-reported subjective knowledge was on average at 2.5 on a 7-point scale. Most knowledge measures were higher in Italy and Germany followed by France and the UK. Moreover, only 16 % of the total sample answered correctly that only farmed fish can be organic.
- About a fifth of the population has actively sought information about organic food, but very few sought information about organic fish or aquaculture. Similarly low numbers were reported for active membership and financial support of environmental organisations. Organic food consumption frequencies showed that the most commonly consumed foods were: vegetables, dairy, eggs, fruit, poultry and beverages respectively. While lower in the frequency list were: red meat, fish/seafood and grains.
- More than half of the participants in all countries reported that the factors that fit the definition of organic fish the most are: 1. No use of toxic chemicals (62 % of the sample) and 2. Natural living conditions (56 % of the sample). Lowest at the ranking order were the factors: farmed in cages at sea (9 % of the sample), minimize use of water (8 %), farmed in a pond on land (8 %), maximize utilization of nutrients (6 %) and minimize escapes (3 %). Most participants considered organic to be an important issue and considered organic fish to have a good quality and to be safe to eat. Organic fish was also considered to be good for consumers' health, good for the environment and contain no additives, but organic fish is reported to be too expensive for consumers' budget.

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- Being organic is not the most important factor defining fish quality in consumer's minds: To define fish quality in general, clean water, followed by fish welfare and feed issues such as balanced feed composition and feed naturally consumed in the wild were considered important. The factors organically produced, ethical slaughtering and wild caught fish were the lowest rated, however still above 5, which is above the 7-point scale's mid-point (i.e. 4).
- Most participants considered organic production to be an important issue and considered organic fish to have a good quality and to be safe to eat. Organic fish was also considered to be good for consumers' health, good for the environment and contain no additives, but organic fish is reported to be too expensive for consumers' budget.
- The respondents were asked which features are relevant in characterising farmed, wild and organic fish. The variables that organic fish was rated lower than farmed and wild fish were: good quality, healthy, animal friendly, environmental friendly, natural, tasty and safe. However, these differences were not large and are expected to be explained by lack of knowledge in the sample
- Consumers are not very active in searching for information about organic fish. The most trusted sources of information were reported to be the organic fish farmers and independent certifiers. Retailers and government were less trusted, with a dichotomy between Italy/Germany and France/UK. The first trusted the governmental controls more than retailers, while the latter the opposite.

A5.2.1. Plans towards deliverable D3.1

The results from the literature review and preliminary findings from the consumer survey were presented in the first stakeholder meeting in October 2014. During this meeting, feedback was given by the stakeholders about topics which are relevant and need to be analysed in depth. This feedback will be taken into account and task 3.1 is continuing with further analysis of the findings. The final output will be delivered on M18 and presented to stakeholders in the second stakeholder event in OrAqua for a final confirmation of its relevance.

A5.3 Economic analysis

- Due to the density limits in the EU-regulation and a more cautious feed strategy the production on farm level will decrease (under the assumption that the production capacity will not change). This is particularly the case for salmon and trout. The maximum density in organic carp, sea bass and sea bream production does not differ from the conventional production systems
- The production costs per kg fish in organic production systems are substantially higher. The calculations show a higher cost price for salmon of 20-30%, for trout 25-40%, for sea bass and sea bream of 20-30% and for carp 10-20%. The main reasons are:
 - Due to the lower production the fixed costs have to be spread over less kg marketed product.
 - The price for feed is 25 to 30% higher
 - Organic raised juveniles are more expensive
 - Relatively more labour is required for e.g. health care (prevention, parasite control)

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- Producing and selling a 'quality product' needs more special attention, marketing skills and risks. More high qualified labour is required.
- Due to the lower production some size-related labour advantages will decrease
- No big differences are expected for:
 - mortality
 - FCR: Since organic and conventional feed are equivalent in the matter of energy content the feed conversion rate can be equal.
- The higher costs compel a higher selling price of the organic fish of 15-30% in comparison to conventional. For other organic products this higher price is indeed a fact. We still have to find out if this higher price is realistic in aquaculture.
- Most of the data are from the STEFC-database. In some cases the data seem incomplete. This may have influence on the results, in particular to the absolute level of the difference of the cost prices of organic and conventional fish. The relative influence is smaller. This problem is still a point of interest.
- The basic assumptions regarding the price and volume mutations changes are mainly based on experiences and knowledge of field experts. These assumptions are being reviewed after input at the stakeholder meeting in Istanbul.
- More information is needed for extra costs in the after-farm part of the chain, like transport, alternative slaughtering methods, packaging and marketing.

A5.4 Institutional frameworks

- Bureaucratic production rules and control provisions is said to be one of the most significant problems with the existing regulation
- The complexity of (national) bureaucracy is said to slow down the transition to certified production
- There is some doubt about the new regulations ability to contribute to the necessary stability of the sector
- Lack of national policy support by national programs for organic aquaculture production
- There is lack of relevant statistics and updated information regarding organic aquaculture, and it is difficult to have a good understanding of the past and current status
- There is a great variation between the countries with respect to standards and certification. These standards often vary significantly from country to country, certifier to certifier, and species to species
- Companies operating in multiple markets must in some cases be certified in several countries, and some have trouble with the conflicting certifications
- The regulations and standards are said to be devised without reference to economic realities
- Private standards are in some cases considered too strict to be economically viable for producers in challenging markets
- Concerns regarding the cost of certification, especially for small-scale aquaculture producers
- Regulations related to feed, density etc. contribute to high costs that reduce the competitive strength
- Current legislative framework and standards are not adapted to all countries and national initiatives are not harmonized
- Lack of knowledge and confusion among consumers about what is organic and what is not

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- Lack of detailed organic production rules for the phase of the life stage between hatching and weaning of juveniles makes it difficult to distinguish organic and non-organic hatcheries
- Organic aquaculture production may be challenged by stricter regulation for ordinary production, which may wipe out some of the differences between organic and ordinary production.

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