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Seventh Framework Programme
Theme [OCEAN.2011-1]
“Innovative Multi-purpose off-shore platforms: planning, design and operation”
Grant Agreement no.: 288710
Start date of project*: 01 Jan 2012 - Duration: 48 month*

IMTA OFFSHORE
(Integrated Multi-Trophic Aquaculture)

Deliverable: D4.4	
Nature of the Deliverable:	Report
Due date of the Deliverable:	M36 – 31/12/2014
Actual Submission Date:	M50 – 12/02/2016
Dissemination Level:	PU
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Version	Date	Revised Pages	Description of Changes
1.0	31.12.2015	-	1st Draft released
1.1	12.02.2016		Final

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1 Introduction

Future expansion of marine aquaculture likely will take place at offshore sites because competition for space with other activities will increase in coastal waters. Besides reducing conflicts over access rights, moving offshore can be seen as a mean to reduce several environmental problems related to coastal aquaculture such as eutrophication following release of soluble nutrients and impact on seabed caused by accumulating particulate waste. Larger depth and higher current speeds will increase dilution of waste in offshore aquaculture and distribute waste over a larger area, thereby increasing the capacity of the benthic and pelagic ecosystem to assimilate the waste. In a Mermaid modelling study benthic and pelagic impacts of identical sized fish farms were reduced between 4 and 8 times both in terms of excess concentrations (in water and sediment) and area cover of these exceedances supporting the assumption that the assimilative capacity to sequester aquaculture waste *is* higher at offshore sites (see D4.7). However, for economic reasons offshore aquaculture facilities need to be much larger than coastal systems, which will increase waste production locally and thereby the pressure on the environment. Therefore, mitigation measures such as implementing Integrated Multi-Trophic Aquaculture (IMTA) in offshore production systems is a theoretical possibility that hitherto has not been tested at full scale.

The report strives to present a balanced and critical review of the current state of marine IMTA building on facts (and not fiction) on 1) the physiological capacities of different species-groups to capture and assimilate dissolved and particulate waste from fish farming, 2) the physical/ hydrodynamic conditions (constraints) allowing (or preventing) waste to be intercepted by lower trophic levels in an IMTA set-up and, 3) results from field IMTA studies.

2 What is Integrated Multi-Trophic Aquaculture?

Intensive marine fish farming gives rise to dissolved and particulate nutrient waste potentially affecting the surrounding pelagic and benthic ecosystems. Main environmental impacts are related to release of nutrients (primarily NH_4^+) to the water column potentially stimulating algal growth and leading to various eutrophication effects. Another type of effects relate to loss of particulate waste including uneaten feed, fecal pellets as well as detached debris from fouling of the net cage structures. High organic loads may deteriorate sediment quality and reduce diversity of the benthic organisms living in sediments.

The magnitude of impacts depends on size of production – that again scale to the nutrient release and waste deposition, the depth and the overall hydrodynamic conditions at the production site. If established at suboptimal farming sites or if improperly managed the environmental impact may become unacceptable (Sará et al. 2006). In some areas and countries the environmental concerns have reached a level preventing further development of fish farming or even closure of fish farming. However, two recent reviews have shown that application of modern management practices have led to significant reductions in environmental impacts of fish farming over the past 20-30 years (Price et al. 2015, Taranger et al. 2015), suggesting that a general environmental concern may not be warranted.

Release of dissolved nutrients to water and deposition of organic material to the sediments can been seen as unnecessary losses of valuable commodities. These losses may be turned into potential resources for other farmed organisms at lower trophic levels, such as seaweed and mussels. A conversion of both particulate waste (i.e. fish feces and unutilized feed) and dissolved nutrients into valuable products and at the same time reduce the main environmental impacts of feed aquaculture seem a genius concept allowing for environmentally friendly production on the one hand side and potential extra income for the additional produce on the other. The concept of recycling nutrients and an integrated farming of organisms representing different trophic levels, IMTA (Integrated Multi-Trophic Aquaculture) builds on an artisan practice used for food production over centuries in “semi-closed” systems such as inland waters and are still used for extensive production in some cultures today.

2.1 Modern IMTA

In most marine IMTA systems predacious (feed) finfish constitute the motor that besides delivering the fundamental cash flow also generates the primary waste streams that fuel 1) primary producers, typically macroalgae serving as extractors of inorganic nutrients excreted from fish; 2) filter-feeders, such as mussels, oysters or clams, that utilize the fine particulate waste from fish production; and 3) deposit-feeders, which consume the fast-settling particulate waste (from fish and/or from bivalves) otherwise accumulating on the seabed (Figure 1). The optimal implementation of extractive components (species, placement, stocking density etc.) depends on a variety of factors, including hydrodynamic condition at production site, life cycles history of the cultured species and their economic value. The extracting species must be able to use the various waste products generated in the farm and the growing cycles of the different IMTA components should be tightly synchronised in time and space. The timing of waste production should match with the growth periods of the extractive species, and the extracting trophic levels should be able to physically intercept the waste being delivered at a rate and in a resulting concentration that are appropriate to ensure an efficient utilization.

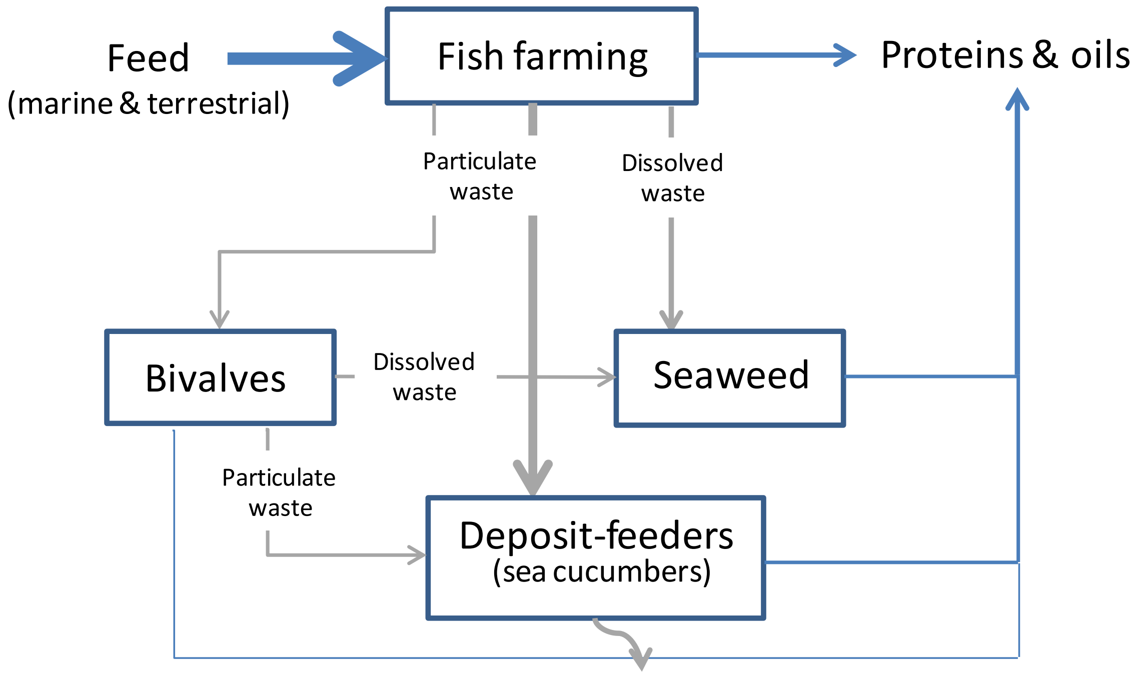

Figure 1 *Conceptual diagram of a "full" marine IMTA system. Thickness of arrows roughly indicates the relative magnitude of carbon flow between trophic levels assuming minimal loss due to dispersion.*

IMTA in coastal waters still is in its infancy and are primarily being tested in non-commercial RDI projects that rarely take account of the proper scales. Lack of solid knowledge are reflected in a large number of SWOT (Strengths-Weaknesses-Opportunities-Threats) analysis carried out by governmental bodies, scientific fora, NGO and others (e.g. Alexander et al. 2014, Bellona 2013, Bolton et al. 2008, Ireland 2015, Slaski et al. 2013, Thomas 2011). MERMAID has attempted to extract and synthesize common information presented in these analyses (Table 1).

Table 1. Simplified SWOT analysis of IMTA

Strengths	**Weaknesses**
• Efficient use of marine space • Recycling of nutrients • Reduced feed demand producing extractive species • Increased crop diversity and overall productivity • Production with a "green" image • Maintain and expand marine activities in rural areas	• Insufficient scientific knowledge leading to a trial-and-error approach • Compromised site selection => sub-optimal conditions (salt, temp, current) for some crops • Difficult business planning (timing of different crops) • Unclear regulatory framework and licensing process • Unknown costumer reaction
Opportunities	**Threats**
• Remediation of eutrophication effects • Potentially increased profitability reducing O&M costs by collaboration between growers • Diversified job opportunities • Eco-food tourism	• Lower productivity than monoculture • Risks for pest transmission between crops • Venture capital and insurance • Negative consumer response to an IMTA-labeling

The Strengths and Opportunities are manifold and generally reflect opinions of scientists, NGOs and governmental bodies that tend to be optimistic on IMTA to solve environmental problems, while aquaculture farmers - typically being in minority or absent in SWOT discussions - focus on the Weaknesses and Threats. The main concerns (Weaknesses & Threats) center on operational issues, including extra costs for setup, construction and maintenance of the extractive production (with unknown market potential), access to capital, licensing issues and consumers reaction – such as “are mussels really raised on fish feces?” (Table 1).With few exceptions, marine IMTA projects and trials have been initiated and organized by scientists focusing on processes and rates, while issues most relevant for farmers such as realistic cost-efficiency of production and market analysis has been lacking. One of the exceptions includes a high-tech integrated IMTA farm being established in Canada (Figure 2).

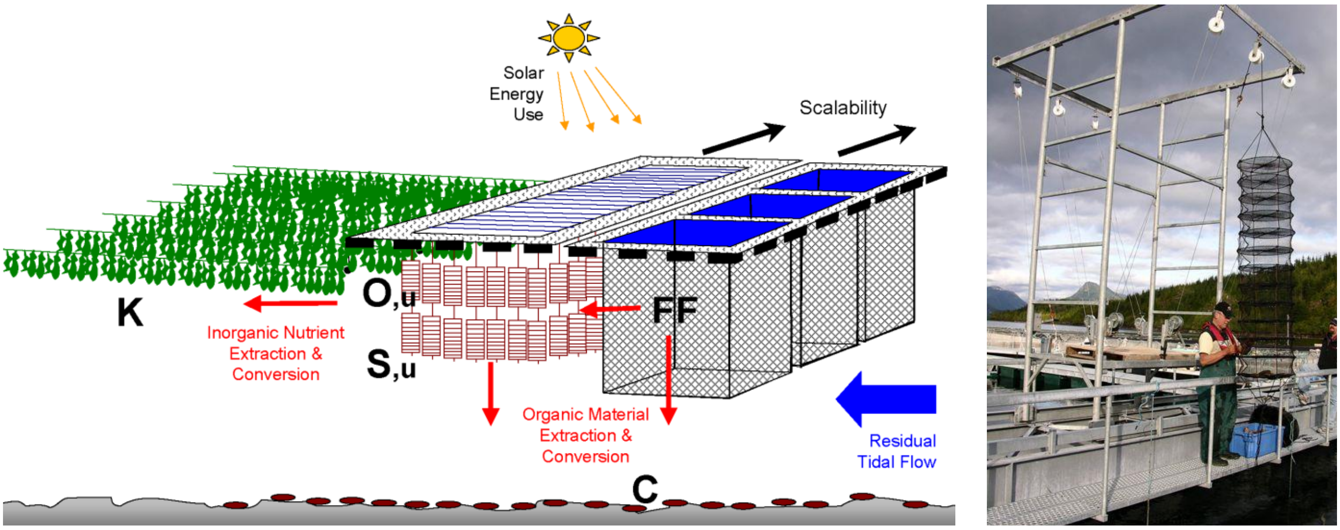

Figure 2 *SEAfood System, Left: schematic drawing showing the placement of different trophic levels (FF: finfish, O: oysters; S: scallops; K: seaweed-kelp; C: sea cucumbers. Right: deploying trays/nets with oysters and scallops. Pictures from Stephen F. Cross, SeaVision Group.*

Most scientific IMTA studies have been limited in scope typically focusing on two trophic levels; either finfish and filter-feeding bivalves or finfish and seaweed. These combinations are discussed below.

3 IMTA – Finfish & Bivalves

Modern finfish farming in marine waters began its expansion in Europe in 1960s and the annual production has now reached 2 mill tons in Europe (430,000 in EU). Five species - in decreasing order - salmon, seabream, seabass, rainbow trout and turbot - dominate the marine production in EU accounting for 85% of the production volume and value. These are also the species dominating the waste-producing trophic level in most IMTA studies. Three species-groups dominate the bivalve production in EU; mussels, oysters and clams with total value at €1.2 billion and thus comparable to the value of fish production. Physiologically, mussels, oysters and clams are rather similar but because of simpler farming method mussels dominate as the “extractive” component in IMTA studies.

3.1 Available food for mussels and oysters

Mussels - and oyster and clams - are generalist consumers of particulate organic matter suspended in the water column. Prime food includes phytoplankton but also microzooplankton and detritus can be ingested and assimilated. Therefore, unlike finfish farming culturing of bivalve mollusks is extensive, and mussels only rely on the natural organic particles in the water and do not require additional (external) food sources. Mussels ingest particles in the size range 1-60 µm with high efficiency (Møhlenberg & Riisgård 1978, Strohmeier et al. 2012), and particles larger than 60-80 µm are rejected. At high particle concentrations (> 2-5 mg suspended solids per L) mussels sort-out inorganic matter and expel this rejected material as “pseudofeces” and preferentially ingest phytoplankton and organic matter (Kiørboe & Møhlenberg 1981).

Compared to suspended particles in the water column, the biodeposits from mussels (feces and pseudofeces) are much larger (mm-size), have much higher settling velocities and requires 5-10 times higher bed sheer stress to resuspend (Chamberlain 2002, Callier et al. 2006). Just as below fish farms sediments under mussel farms may become enriched with organic matter from settling biodeposits, but deposition rates are typically much lower than below fish farms and the mussel waste will resuspend at lower bed shear stress.

3.2 Particulate waste from fish farms

The particulate waste from fish farming consists of a mixture of unconsumed fish feed - typically accounting for 2-3% of total particulate waste loss in well-managed farming - and feces comprising 97-98%. The vast majority of particulate waste consists of large-sized particles (> 100 µm) outside the consumable range of mussels and with settling velocities in the range 1-10 cm/s (Chen et al. 1999, Cromey et al. 2002, Chen et al. 2003, Moccia et al. 2007, Unger & Brinker 2013, Law et al. 2014). In effect, the capture efficiency of waste by mussels deployed near fish farms would be low, because 1) of short residence time of particles within the mussel farm (Cranford et al. 2013, DFO 2013) and, 2) only a small fraction of waste is available to mussels (see above). Residence time of water passing the fish farm can be increased by extending the length of the mussel farm but the concentration of particulate waste will steadily decrease due to sedimentation and dispersion and mussels 500-1000 m downstream the fish farm will not be exposed to waste particles in significant concentrations (Reid et al. 2008). In one study the excess particulate organic matter fell to ambient levels 10-50 m from a salmon farm (Lander et al. 2013).

The nutritional value of fish feed and feces have been studied in under controlled laboratory conditions. The content of carbon and nitrogen was much lower in salmon feces than in salmon feed (Wang et al. 2013) indicating a lower nutritional value of feces-waste than of feed-waste. However, information on *realized* assimilation differs. In one study the assimilation efficiency in mussels exposed to suspended organic carbon from salmon feed or from salmon feces was equally high (Reid et al. 2010). In contrast, another study showed that mussels fed a mixture of fish feed and the flagellate *Rhodomonas baltica* increased their biomass and shell length, while shell length was unchanged and increase in biomass was significantly lower in mussels fed a mixture of fish feces and *R. baltica* (Handå et al. 2012).

3.3 Fate of waste in the water column

The behavior of waste particles from a 3,000 tons rainbow trout farm (Musholm West) located in the Great Belt, Denmark, was described using a numerical model (MIKE3-FM) by applying different settling velocities (0.1 mm/s, 1 mm/s and 10 mm/s) to 3 size groups of particles. Particles were released in the upper 0-4 m and horizontally distributed evenly over the 200m * 200m farm center. Hourly current speed in the upper 0-7 m varied between 2 and 38 cm/s with northbound currents in 68% of time. Bottom water (8-14 m) current speeds varied between 1 and 22 cm/s with southbound currents in 73% of the time. Particles in the size range 5-15 µm with a mean settling velocity of 0.1 mm/s were mainly distributed north of the fish farm with the highest concentrations in surface waters (Figure 3), but 350-500 m north of the farm center concentrations of the small-sized waste was reduced to 1/20 due to horizontal dispersion (coefficient $Kx = 2\ m^2/s$) and sedimentation. The horizontal distribution was even more restricted for the larger size fractions (Figure 3 E & G).

The model study accentuates the almost impossible odds that IMTA farmers face when using mussels to sequester particulate waste and reduce benthic impacts; 1) the vast majority of particulate waste occurs in a size range too large to be ingested by mussels and 2) only a small fraction of the waste particles available to ingestion will be intercepted by mussels, because waste residence time within the farm is low.

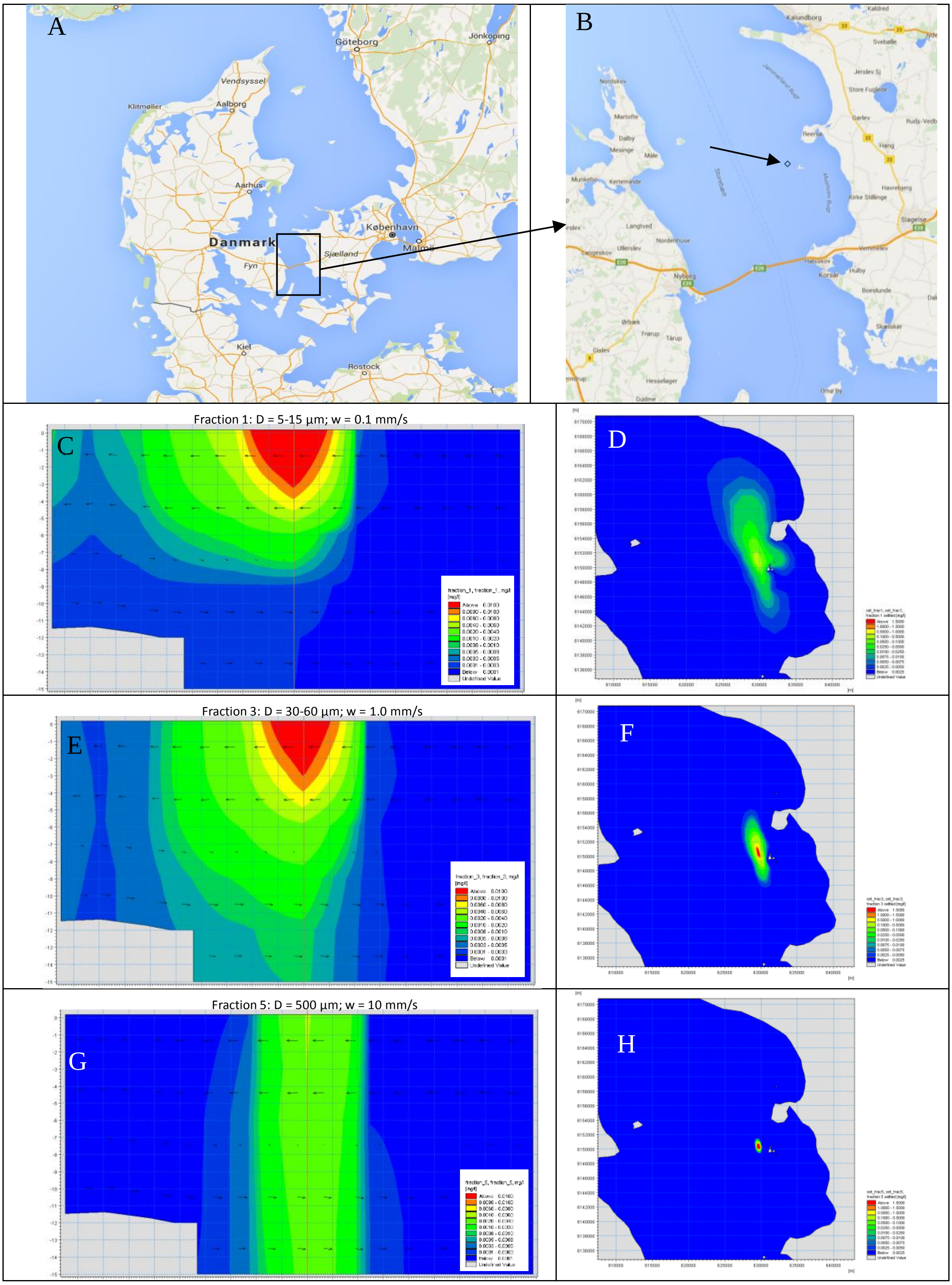

Figure 3 *Modelled distribution of particulate waste from a 3,000 tons rainbow trout farm in the Great Belt, Denmark. A & B: location of farm - arrow; C, E & G: average (2 d simulation) concentration of 3 size groups of waste in a 1,000 m long vertical (N-S) section; D,F & H: distribution of sedimented waste (3 size groups) on seabed after 14 days simulation. Current vectors shown in C, E & G.*

The scientific literature is inconclusive about the applicability of integrated fish-bivalve culture. Various studies have shown that mussels assimilate organic matter from particulate waste or mussels/oysters increased growth when deployed near a fish farm (e.g. Chopin et al. 2008, MacDonald et al. 2011, Rensel et al. 2011, Lander et al. 2012, Aguado-Giménez et al. 2013), while other scientists have not found evidence for that particulate waste will stimulate growth of mussel and oyster or contribute significantly to their diet (Parsons et al. 2002, Cheshuk et al. 2003, Navarrete-Mier et al. 2010, Rensel et al. 2011). In accordance with spatial variation in waste concentration positive effects such as increased growth or accumulation of fish-feed tracers (e.g. fatty-acids) in mussels are exclusively (?) reported for mussels or oysters deployed next to fish farms, while those who studied mussels placed at larger distance from fish farms (e.g. Irisarri et al. 2014) did not find differences in mussel growth at a site 170 m from a seabream farm and at a reference site 550 m from a fish farm.

To conclude from available information (cited above):

- Growth of filter-feeding bivalves such as mussels and oysters *may* benefit from additional food sources consisting of particulate waste from fish farms especially during periods of low phytoplankton concentration
- Only bivalves located close to fish farms may take advantage of waste – at larger distance from fish farms waste concentration is too low
- In open waters farming of mussels and oysters will not be able to sequester more than 1-2% of the particulate waste released from a fish farm.

4 IMTA – Finfish & Seaweed

In recirculated waters (recirculation aquaculture systems - RAS) and in semi-closed systems cultivation of aquatic plants such as macroalgae has been a very successful mean to assimilate inorganic nutrients released from finfish farms and thereby recirculate nutrients and mitigate environmental impacts caused by nutrients (e.g. Neori et al. 1998, Turcios & Papenbrock 2014). In open systems, e.g. marine coastal and off-coastal waters the use of seaweed to sequester dissolved nutrient released from fish farms is less straightforward because environmental conditions cannot be controlled.

Nutrients and light are the most important environmental factors controlling growth and biomass yield in seaweed farms. Except for few environments (Eastern Mediterranean Sea, Northern Baltic Sea) nitrogen rather than phosphorus is the main production-limiting nutrient and will be used as a proxy for “nutrients” in this section. Nitrogen in form of NO_3^-, NH_4^+ and urea are the main nitrogen species available for seaweed. Concentration of these species in marine wasters varies with geographical location and season, but concentrations are typical low in tropical seas year around and during late spring – through to autumn in temperate seas. Near to specific nutrient sources (sewer outlets, river mouths, upwelling areas) nitrogen concentrations can be higher and allowing seaweed growth during otherwise nutrient depleted periods.

4.1 Seaweed and nitrogen

All nutrient uptakes in seaweed take place from water; nitrate by an active (energy consuming) process, ammonia and urea partly by passive (diffusion) processes (Hurd et al. 2014). Generally, the nutrient uptake capacity is higher than needed for maximal growth; hence, during nutrient replete periods seaweed can accumulate high nutrient concentrations in their tissue and use this "storage" for growth during nutrient depleted periods. The efficiency of nutrient uptake from water differs between species and taxonomic group (class). Important parameters expressing the efficiency include the maximal uptake rate V_{max}, the ambient nitrogen concentration K_s where ½ * V_{max} is reached and, the ratio α - expressed by V_{max}/K_s. α reflects the relative efficiency of nitrogen uptake at ambient (low) concentrations. Within classes (green, red and brown algae) the relative efficiency of ammonia uptake is higher than uptake of nitrate (α is 2-3 timer higher), and between groups the half-saturation constant, K_s is lowest for brown algae implying that this group are best adapted to grow at relatively low nitrogen concentrations (Table 2). Green algae have very high V_{max} for NH_4 uptake (≈ 100) reflecting a large capacity for "surge" uptake under fluctuating nitrogen concentrations.

Table 2 Summary statistics of kinetic constants (Ks and α = Vmax/Ks) for uptake of ammonium and nitrate in green, red and brown macroalgae. Median and range values (in brackets) shown. Based on data from Rees (2003) and Hurd et al. (2014).

	Green		**Red**		**Brown**	
	Ks (µM)	α (V_{max}/K_s)	Ks (µM)	α (V_{max}/K_s)	Ks (µM)	α (V_{max}/K_s)
NH_4	15.5 (5 - 48)	9.4 (0.1 - 18)	16.9 (2.5 - 76)	2.8 0.1 - 22)	4.8 (3.5 - 40)	6.9 (0.2 - 18)
NO_3	5 (3 - 26)	4 (2 - 40)	6 (2.5 - 19)	1.4 (0.2 - 6)	6.8 (0.5 - 25)	2.4 (0.2 - 11)

4.2 Release of ammonia from fish farms

In addition to particulate waste fish farms release dissolved nitrogen (primarily NH_3 that dissociate to NH_4^+) through excretion. Typically, in well-managed fish farming using optimised feed allowing for high feed-conversion about 45% of feed nitrogen is lost/excreted as NH_3 (Wang et al. 2012). Other authors calculate higher release rates of dissolved nitrogen (Norði et al. 2012). Despite high losses significantly elevated nitrogen concentrations near fish farms are rarely seen. In a scoping study to examine possibilities for seaweed farming Sanderson et al. (2008) found elevated NH_4^+ concentration (ca. 2 µM above background) in the lee side of a salmon farm in Scotland. Price and Morris (2013) reviewed 21 recent publications; in 5 of these nitrogen was significantly elevated, in 7 studies (including a comprehensive study collecting and analysing 25,000 water samples) increase in nitrogen could not be documented and, in 9 studies impact levels was evaluated being minimal - authors reported elevated concentrations that either were non-significantly above background concentrations or were not thought to have significant environmental implications. In their review, Price and Morris (2013) concluded that when occurring, elevated nitrogen in the water column around fish farms is typically a localized effect (within a hundred meters), often with seasonal variation. Farms located in deep well-flushed waters rarely will give rise to water quality impacts.

4.3 Can fish farms supply sufficient nutrients to grow seaweed cost-efficiently

Using mass-balance approaches, i.e. matching release rates of nutrients from fish farms with the elementary C:N:P ratios in various seaweed species several studies have estimated the theoretical capacity for seaweed to sequester nutrients released from fish farms (e.g. Reid et al. 2013, Reida et al. 2013, Wang et al. 2012, Wang et al. 2013). Without taking account of uptake kinetics in seaweed (see Table 2) and dilution rate of excreted nutrients such approaches invariable will overestimate the farming potential of seaweed.

Several studies have measured growth rate of seaweed deployed at small scale adjacent to fish farms and compared growth and/or nutrient accumulation with seaweed deployed at reference sites. In most studies growth rate and yield higher were higher in the vicinity to farms (Chopin et al. 2004, Buschmann et al. 2008, Abreu et al. 2009, Sanderson et al. 2012, Handå et al. 2013) than at reference sites, but very few studies (e.g. Abreu et al. 2009) have presented qualified predictions on the area and distance from a fish farm that can support enhanced growth of seaweed.

Yearly growth-bioassays with the green macroalgae *Ulva lactuca* held in small transparent tubes (10 cm diameter) deployed at different distances from a 3,000 tons fish farm (see Figure 3 for location) showed that macroalgal growth was stimulated up to 500 m from the fish farm, however, with a marked decrease with increasing distance (Figure 4). The estimated enrichment of water passing through the farm with NH_4^+ was estimated to 1.5-2 µM. The decrease in realised growth rate in *Ulva* paralleled NH_4^+ predictions from numerical modelling and is explained by dilution and uptake in phytoplankton. At low nitrogen enrichment levels phytoplankton will outcompete most seaweed species because phytoplankton have lower Ks and higher α (Hein et al. 1995).

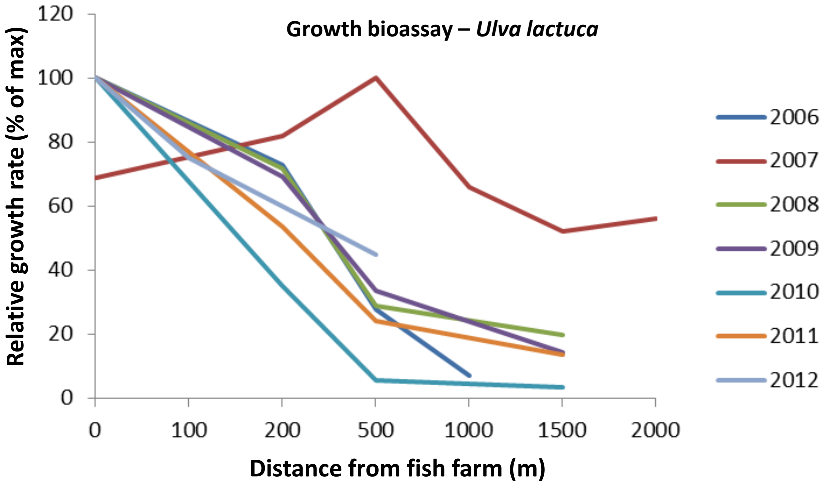

Figure 4 Growth response of Ulva lactuca deployed – in triplicate - at increasing distance (north of fish farm along the dominant current direction). Max growth rates varied between 0.08 – 0.26 d^{-1} (average 0.18). All bioassays were carried out in September when feeding intensity and NH_3 excretion was maximal (≈ 325 kg NH_3-N). Data from DHI (2013). Bioassay is detailed in Lyngby & Mortensen (1994).

A recent numerical modelling study and a full-scale integrated fish farm–seaweed culture confirm that assimilation of dissolved nitrogen loss from fish farms into seaweed is very inefficient (less than 1% of N-loss assimilated) and that nutrients released from fish farms contribute insignificantly to support seaweed growth at well-flushed aquaculture sites (Broch et al. 2013, Marinho et al. 2015).

To conclude from available information (cited above):

- At well-flushed open water aquaculture sites (mean current speeds ≥ 10 cm/s) NH_3-excretion from farmed fish will not lift available nitrogen to concentration levels that can stimulate growth of seaweed significantly
- In well-flushed open water aquaculture areas seaweed culturing will not be able to sequester more than few percentage of the dissolved nitrogen released from nearby fish farms.

5 IMTA – Finfish & deposit-feeders

In open water aquaculture IMTA is hampered by high dilution rate of soluble waste from fish farms preventing cost-efficient sequestering of nutrients by seaweed farming and, the size distribution of particulate waste (unconsumed fish feed and feces) and high settling velocity makes the majority of solid waste unavailable for mussels and oysters.

Deposit-feeders such as sea urchins and sea cucumbers are more effective to sequester the organic material settling below fish farms, but their incorporation in open water IMTA is at a very early (research) stage. Under laboratory conditions sea cucumbers are efficient converting organic waste into biomass (MacDonald et al. 2013), but field tests are required to test their behavior and efficiency under natural conditions, e.g. according to Hamel & Mercier (2008) growth rate of the native North European sea cucumber *Cucumaria frondosa* is low.

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