

Nutrient uptake and growth of Irish Moss (*Chondrus crispus*, Rhodophyta) in dilute fish effluent

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Abstract

Irish Moss (*Chondrus crispus*) was grown successfully outdoors for 27 days in 20-litre containers in the diluted effluent from tanks containing sea trout (*Salmo trutta trutta*). Growth and nutrient uptake were compared in containers receiving either a continuous flow of natural seawater or effluent from the fish-cultivation tank, and a parallel batch experiment was set up in which the seawater and effluent were changed every second day. The nutrients were taken up by the seaweed with removal efficiencies in the fish effluent flow-through culture system of 100 %, 34 %, 53 % and 50 % for NH_4^+ , NO_3^- , NO_2^- and PO_4^{3-} , respectively. Specific growth rates (SGR) of 0.03 d^{-1} were reached in the two flow-through culture systems. At the end of the experiment, *C. crispus* receiving a continuous flow of fish effluent had N-content higher (2.5 % N of DW) than that in the other three treatments with values below the critical level of 2 % N of DW. However, thalli became bleached only in the two batch cultures, suggesting N-limitation and therefore utilization of the nutrients bound in the phycobilins. Extrapolation of biomass increment during this short experiment to a full year gave an annual production of $14 \text{ kg DW m}^{-3} \text{ y}^{-1}$. The simple set-up of this experiment, without extra addition of nutrients, CO_2 or artificial light, showed that *C. crispus* could reduce nutrient from the fish effluent noticeably and reach SGR and production comparable to that in laboratory and outdoor tank experiments with more elaborate equipment and greater running costs. Nevertheless, further investigation is needed to improve our understanding of this candidate for bioremediation, and whether this nutrient conversion is profitable, or an environmental cost due to future regulations on nutrient rich effluent.

Key words: Ammonia, N-content, nitrate, N:P ratio, phosphate, specific growth rate

1. Introduction

Irish Moss (*Chondrus crispus* Stackhouse) is a temperate species of red seaweed native to Europe and the Atlantic coast of North America (Rosenvinge, 1931; Pringle and Mathieson, 1986; Maggs and Hommersand, 1993) and natural populations have long been harvested from rocky shores or collected from storm drift because it contains the polysaccharide carrageenan, used as a gelling agent in food, toothpaste and pharmaceutical products, etc. (Pringle and Mathieson, 1986; Bixler, 1996). Because of this valuable polysaccharide, the growth rate and carrageenan content, temperature and nutrient preferences of *C. crispus* as well as its potential for inland and off-shore commercial production have been extensively examined and are well documented (Neish et al., 1977; Simpson and Shacklock, 1979; Bidwell et al., 1985; Amat and Braud, 1990; Juanes and McLachlan, 1992; Kübler and Davison, 1993; Chopin et al., 1995; Braud and Amat, 1996; Chopin et al., 1999; Chopin and Wagey, 1999; Zertuche-González et al., 2001).

Chondrus crispus is known to grow more slowly than other seaweeds that also contain carrageenan, such as *Eucheuma denticulatum* and *Kappaphycus alvarezii*, which are cultivated commercially in tropical waters, but the value of *C. crispus* is higher, because of its greater carrageenan yield, which compensates for the lower specific growth rate (Chopin et al., 1999).

Seaweeds can be used in marine aquaculture systems which integrate fed and extractive organisms such as fish and seaweed, respectively. The seaweed assimilates the dissolved inorganic nutrients from the water, acting as a biofilter and turning wastes into valuable biomass (Cohen and Neori, 1991; Buschmann, 1996; del Rio et al., 1996; Bodvin et al., 1996; Schuenhoff et al., 2003; Neori et al., 2003; Schuenhoff et al., 2006; Hernandez et al., 2006). Fish excretion supplies ammonia-N ($\text{NH}_3 + \text{NH}_4^+$), dissolved organic nitrogen (DON) and faeces-N to the effluent, and the DON is rapidly transformed into ammonia-N and all inorganic N nutrients ($\text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-$) are available for assimilation by seaweeds (Krom et al., 1995; Cowey, 1995; Neori, 1996; Harrison and Hurd, 2001). Phosphorus is also excreted by fish as phosphate (PO_4^{3-}), dissolved organic phosphate (DOP) and particulate organic P, and up to half of the P in trout faeces may be available for algal production (Dosdat et al., 1995; Neori, 1996).

Significant trapping of these nutrients by *C. crispus* may increase the production of biomass and thus convert excess nutrients into a valuable product, such as carrageenan, and will also reduce the negative ecological effects of excess nutrient loads (Ellner et al., 1996; Neori et al., 2004).

This investigation examines the nutrient uptake of *C. crispus* in a simple set-up using fish effluent as nutrient source. In an outdoor experiment, *C. crispus* was grown for 27 days in containers receiving a continuous flow of either natural seawater or fish effluent. A parallel batch experiment was set up in which the seawater and fish effluent were changed every second day. The specific growth rate, nutrient composition and nutrient uptake of *C. crispus* in the four different treatments were investigated together with the extent of epiphytism. The potential of this valuable seaweed as bioremediator in land-based fish farms is discussed.

2. Materials and methods

2.1. Algal material

Non-reproductive specimens of *Chondrus crispus* (life history stage not determined) were collected from an intertidal rocky shore near Portaferry, Northern Ireland (54°22.6' N, 5°33.2' W) during February and March 2005. A total of 175–178 individuals (4–10 cm; dichotomous to branched - class II and III; Pringle and Mathieson, 1986) with an initial wet weight (WW) of 0.059 kg were placed in each of four containers (plant density 2.95 kg WW m⁻³).

2.2. Culture conditions

Transparent 20-litre plastic containers with transparent lids (height: 22 cm; length x breadth: 33 x 42 cm at top, 27.5 x 37 cm at base) received a continuous flow of either natural seawater or effluent from the fish-cultivation tank growing sea trout (*Salmo trutta trutta*). A parallel batch experiment was set up in which the seawater and effluent were siphoned and exchanged 100 % every second day. The water in each container was aerated and mixed by U-shaped plastic pipes (PVC-electrical pipes, 16 mm diameter) located 1 cm above the container base with holes drilled horizontally (1 mm diameter, 35 cm intervals). The rate of air flow in each container was 20 L min⁻¹.

The two flow-through containers had an overflow covered with mesh to prevent loss of biomass. The rate of water flow in these containers was adjusted every

second day to 0.375 L min^{-1} (equal to 27 water exchanges d^{-1}). Fish effluent from the sea trout (*Salmo trutta trutta*) cultivation tank was channelled into a settling tank, which provided water for both flow-through and the batch fish effluent tanks. In the flow-through tank, this resulted in daily variations in nutrient concentrations, because of daily feeding routines.

The containers were located outside at ambient temperatures and natural light, and the experiment was conducted from 26 March to 22 April 2005. Water temperature in the containers was measured twice a day over a two-day period at the end of the experiment. The pH was measured in water samples at randomly chosen days.

2.3. Growth measurements

In each tank, 15 individual thalli were tagged with a coloured PVC-free plastic bead (Hama midi-bead: length 5 mm, diameter 5 mm) tied with nylon string (fishing line 0.35 mm) and used for growth measurements for each week of experiment. During the experiment some tagged thalli either lost their marker bead or some part of the thallus broke off (the latter especially in the batch cultures), and these were not included in the estimates of total biomass or specific growth rates of the tagged individuals.

Blotted wet weight (WW) of the tagged individuals of *C. crispus* was measured on days 7, 13, 22 and 27, and the weekly specific growth rate was calculated from initial wet weights (t_0) and the consecutive weekly wet weights (t_w) using the formula:

$$\text{Specific Growth Rate} = \text{SGR} = (\ln (\text{WW}t_w) - \ln (\text{WW}t_0)) / (t_w - t_0)$$

Total SGR was calculated using the same formula, but on basis of the total biomass increment with total initial wet weight ($\text{WW}t_0$) and the total final wet weight biomass ($\text{WW}t_w$) after the 27 days of experiment.

2.4. Tissue and water samples

Tissue of *C. crispus* was sampled at the start and at the end of experiment from the tagged biomass. Tissue samples were dried and boiled in 40 mL distilled water for 20 min and extracts were frozen until analysis. The daily water samples were also frozen until the concentrations of NO_3^- , NO_2^- , NH_4^+ and PO_4^{3-} were estimated using an

AutoAnalyzer 3 (Bran + Luebbe). Every sample was analyzed twice, and the averages are presented. Nutrient concentrations are presented as μM but can be expressed in mg L^{-1} by multiplication with conversion factor (molar weight) 0.062 for NO_3^- , 0.046 for NO_2 , 0.018 for NH_4^+ and 0.095 for PO_4^{3-} .

The average dissolved inorganic nitrogen (DIN) in the water flowing through the culture tanks was calculated as the mean of the total nitrogen concentrations (NH_4^+ , NO_3^- and NO_2) in the inflow and outflow during the experiment. Because the concentrations of DIN on day 2 in the batch cultures were much lower than on day 1, the mean values of DIN throughout the experiment were calculated as the mean of total nitrogen concentrations in fresh, 1-day-old and 2-day-old water.

Tissue samples of *C. crispus* (five pieces of thalli from each treatment) at the end of the experiment were also analyzed for carbon, nitrogen and sulphur content (expressed as % of DW) using a CHNS analyzer (CE instruments EA 1110). Sulphur content was estimated because carrageenan is a sulphated polysaccharide (McCandless and Craigie, 1979; Roberts and Quemener, 1999).

Thalli representing the colour of the biomass in the different cultures were used for colour estimation in Jasc Paint Shop Pro 8. A colour scale of red, green and blue was used to find the colour match.

All results are presented as average \pm standard deviation.

3. Results

3.1. Physical factors: pH, salinity and temperature

During the 27 days of experiment, the pH was 8.8 ± 0.2 with no significant difference between the inflow and outflow water. Salinity ranged from 30 to 33‰ during the experiment. The temperature in the flow-through cultures was 12.0 ± 1.4 °C, whereas the batch cultures fluctuated with the ambient temperature (day temperature 10–18 °C) with a mean of 13.0 ± 3.8 °C.

3.2. Colour

After 27 days, the colour of the cultured fronds of *Chondrus crispus* was clearly different in the batch cultures and in the flow-through systems. The algae in the flow-through systems with both seawater and fish effluent were dark red while those in the batch cultures were pale greenish in colour at the end of the experiment (Figure 1). The colour analysis suggested that both red and blue had decreased in the batch

cultures, whereas the intensity of green was similar. This change to a paler green colour for the plants in the batch cultures was first recorded after 13 days of experiment.

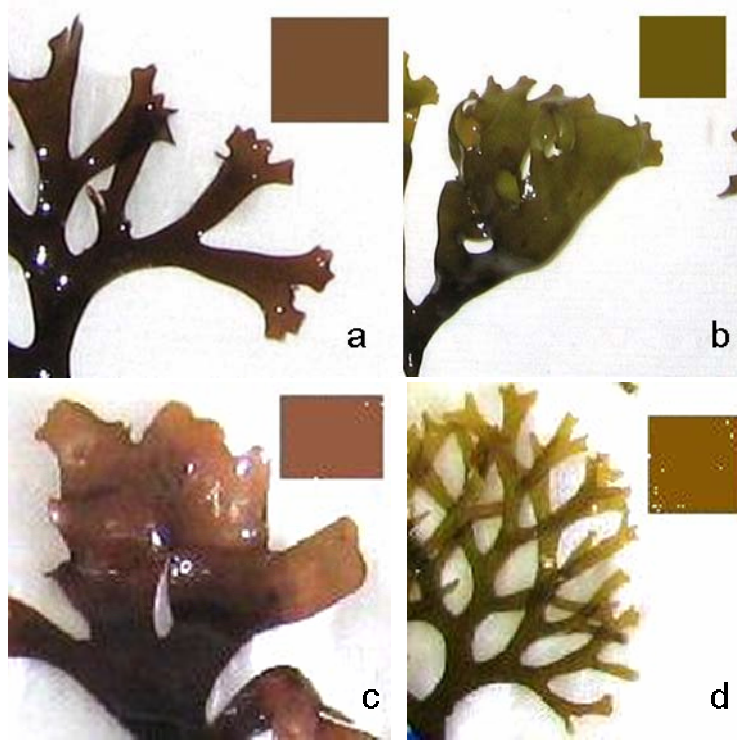


Figure 1. Representative thalli of *Chondrus crispus* after 27 days culture in (a) flow-through fish effluent, (b) batch fish effluent, (c) flow-through seawater and (d) batch seawater. Inserted rectangles represent colour scale: (a) Red: 81, Green: 54, Blue: 34, (b) R: 66, G: 54, B: 9, (c) R: 88, G: 54, B: 34 and (d) R: 76, G: 52, B: 9.

3.3. Biomass and specific growth rate

The seaweed grew successfully in all containers and exponential growth curves could be fitted to weekly measurements of total biomass of the tagged thalli in the flow-through systems, whereas linear equations fitted best growth of the batch cultures (Figure 2).

The SGR of the tagged *C. crispus* was $0.029 \pm 0.007 \text{ d}^{-1}$ in the flow-through effluent, $0.025 \pm 0.008 \text{ d}^{-1}$ in the flow-through seawater, $0.012 \pm 0.004 \text{ d}^{-1}$ in the batch effluent and $0.014 \pm 0.004 \text{ d}^{-1}$ in the batch seawater cultivation system during the 27 days. This resulted in biomass increments of 110 % and 50 % with biomass of around 125 g WW and 90 g WW for the flow-through and batch cultures, respectively, by the end of the experiment.

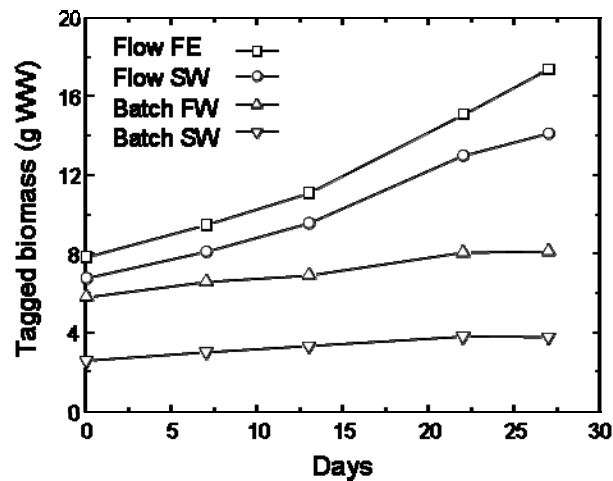


Figure 2. Total biomass (g WW) per tank of tagged *Chondrus crispus* measured weekly in the four different treatments ($n=6-14$). FE: fish effluent, SW: seawater. Linear and exponential fits to the growth data were: Flow fish effluent: ExpGro, R^2 : 0.9987; flow seawater: ExpGro, R^2 : 0.9917; Batch fish effluent: Linear fit, R^2 : 0.9746; Batch seawater: Linear fit, R^2 : 0.9600.

The flow-through culture containers and the mesh preventing the loss of biomass were cleaned of epiphytes (mainly *Ectocarpus* sp.) on day 21, but there was no significant epiphyte growth on the *C. crispus* plants in any of the treatments.

3.4. Nitrate (NO_3^-)

Over the 27 days of the experiment, the average NO_3^- concentrations in the inflow to the flow-through tanks were $0.84 \pm 0.22 \mu\text{M}$ and $0.76 \pm 0.24 \mu\text{M}$ in the seawater treatment and they followed a similar pattern since fluctuations in the NO_3^- concentrations of the natural seawater were reflected in both inflows. The NO_3^- concentrations of the water flowing out of these two treatments were similar with an average concentration of $0.52 \pm 0.21 \mu\text{M}$ (Figure 3). There was a reduction in NO_3^- concentration between the inflow and outflow in flow-through cultures with both fish effluent (37 %) and seawater (31 %; Figure 3).

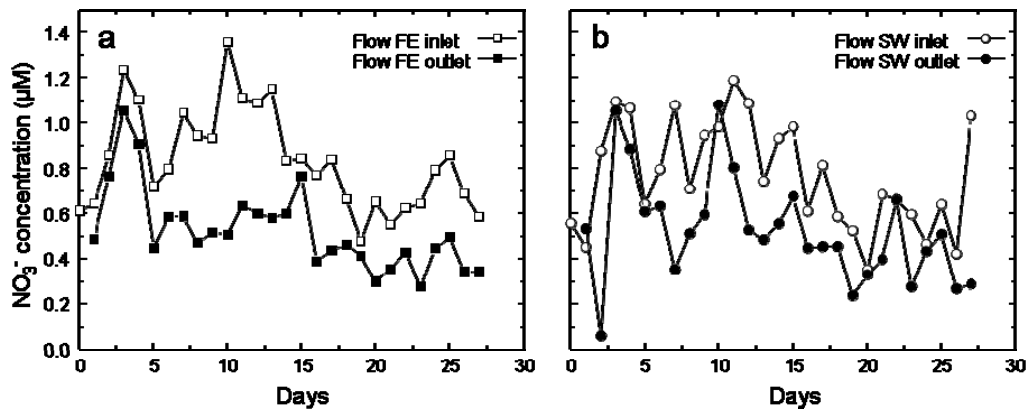


Figure 3. Nitrate (NO₃⁻) concentrations (µM) over 27 days in the inlet and outlet water of flow-through cultures with *Chondrus crispus* receiving (a) fish effluent (FE) and (b) seawater (SW).

Nitrate concentrations in the fresh (i.e. just changed) were on average 0.81 ± 0.17 µM and 0.77 ± 0.20 µM in the fish effluent and seawater batch cultures, respectively and the concentrations in the 2-day-old water were 0.43 ± 0.26 µM and 0.40 ± 0.21 µM in the fish effluent and in the seawater treatment, respectively. There was a 40 % removal of NO₃⁻ in both of the batch cultures on the first day and on the second day 10 % and 16 % in the batch fish effluent and seawater, respectively after each water change (Table 1). The total removal of NO₃⁻ over 2 days between the fish effluent and the seawater treatments was on average 46 %.

Table 1. Percentage removal of nutrients (average \pm SD) in flow-through and batch cultures with *Chondrus crispus* over 27 days.

Nutrient	Treatment	Cultures			
		Flow-through Inflow-Outflow	Batch cultures		
			Day 1	Day 2	Both days
NO ₃ ⁻	Fish effluent	37 \pm 15 %	40 \pm 26 %	10 \pm 14 %	44 \pm 24 %
	Seawater	31 \pm 24 %	40 \pm 19 %	16 \pm 29 %	48 \pm 22 %
PO ₄ ³⁻	Fish effluent	50 \pm 22 %	57 \pm 26 %	25 \pm 23 %	67 \pm 19 %
	Seawater	36 \pm 20 %	57 \pm 24 %	16 \pm 21 %	66 \pm 8 %
NO ₂	Fish effluent	58 \pm 35 %	62 \pm 40 %	26 \pm 35 %	58 \pm 40 %
	Seawater	48 \pm 35 %	55 \pm 24 %	46 \pm 33 %	56 \pm 46 %

3.5. Nitrogen dioxide (NO₂)

Concentrations of NO₂ in the flow-through systems followed the same patterns with fluctuations in the natural seawater also reflected in the fish effluent and with an average of 0.14 ± 0.10 µM in the fish effluent inflow and 0.11 ± 0.10 µM in the seawater inflow. The removal of NO₂ was 58 % and 48 % in the fish effluent and seawater flow-through systems, respectively (Table 1).

The NO₂ concentrations in the freshly changed water of both the fish effluent and the seawater batch cultures were 0.12±0.11 µM and 0.11±0.10 µM, respectively, whereas NO₂ concentrations in the 2-day-old water from the fish effluent treatment were 0.04±0.10 µM and seawater treatment were 0.03±0.10 µM on average. As with NO₃⁻, the NO₂ concentration decreased between water changes in both the effluent and the seawater batch cultures (Table 1). The total NO₂ removal in the two batch cultures was 56–58 %.

3.6. Ammonium (NH₄⁺)

Ammonium was detectable only in water samples from the inflow of the flow-through fish effluent culture or immediately after changing water in the fish effluent batch culture when the mean concentration was 0.70±0.55 µM over the 27 days of the experiment. In all other water samples, NH₄⁺ concentrations were below the detection limit.

3.7. Phosphate (PO₄³⁻)

The concentrations of PO₄³⁻ in the inflowing water were 1.20±0.26 µM in the fish effluent and 0.77±0.25 µM in the seawater treatments, and the concentrations of the outflowing water was 0.61±0.32 µM and 0.51±0.23 µM in the fish effluent and seawater, respectively. The reductions in PO₄³⁻ concentrations during passage through the flow-through cultures were 50 % for the fish effluent and 36 % for the seawater treatment (Table 1).

The mean PO₄³⁻-concentration of the freshly changed water over 27 days in the fish effluent batch culture was 1.04±0.28 µM and 0.73±0.14 µM in the seawater treatment, and the concentrations in the 2-day-old water were 0.31±0.14 µM and 0.24±0.05 µM in the fish effluent and the seawater batch cultures, respectively (Figure 4). The average removal of PO₄³⁻ over 2 days was 66 % in both the fish effluent and the seawater treatments; however the largest removal of PO₄³⁻ occurred on the first day (Figure 4, Table 1).

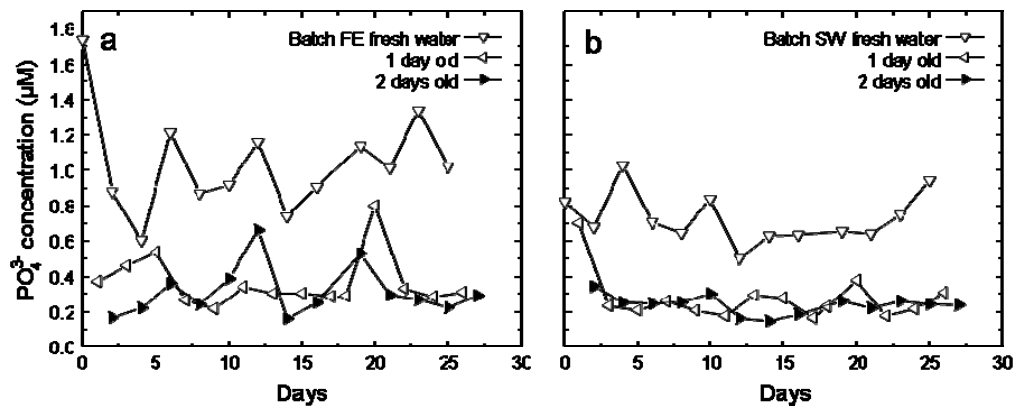


Figure 3. Phosphate (PO_4^{3-}) concentrations (μM) over 27 days in the inlet and outlet water of flow-through cultures with *Chondrus crispus* receiving (a) fish effluent (FE) and (b) seawater (SW).

3.8. Nutrient balance

The carbon contents of *Chondrus crispus* at the end of the experiment were 30–32 % among the four treatments and with sulphur contents of 4–5 % of dry weights (Table 2). Nitrogen content of thalli from the fish effluent flow-through culture was 2.5 % and 1.6–1.9 % of dry weights in thalli from the three other treatments (Table 2). Consequently, the C:N ratio of thalli from the fish effluent flow-through treatment was lower than that of thalli from the other treatments (Table 2).

Table 2. Carbon, nitrogen and sulphur content (% of DW \pm SD) of *Chondrus crispus* after cultivation for 27 days in four different treatments ($n=5$).

Treatment	Tissue content			
	Carbon %	Nitrogen %	Sulphur %	C:N ratio
Fish effluent flow	29.5 \pm 1.2	2.5 \pm 0.2	5.0 \pm 1.4	14
Seawater flow	29.7 \pm 0.8	1.9 \pm 0.2	4.2 \pm 2.1	18
Fish effluent batch	30.3 \pm 1.0	1.7 \pm 0.4	4.3 \pm 2.3	21
Seawater batch	31.6 \pm 2.1	1.6 \pm 0.4	4.0 \pm 1.3	23

There was a positive relationship between the dissolved inorganic nitrogen (DIN) in the water for the four different treatments and the nitrogen concentration of the thalli at the end of the experiment (Figure 5). Highest DIN values were found in thalli (2.5 % of DW) as well as water (1.1 μM) from the flow-through system receiving fish effluent. Lowest DIN values for thalli and water samples were found in the seawater

batch culture (0.7 μM and 1.6 % of DW). However, there was no relationship between the SGR of the tagged *C. crispus* and DIN (data not shown).

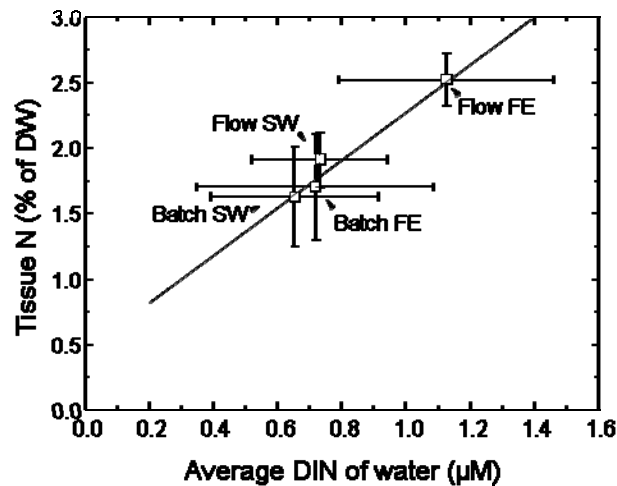


Figure 5. Concentration of nitrogen in thalli of *Chondrus crispus* (tissue N % of DW) at the end of the experiment plotted against average dissolved inorganic nitrogen (DIN, μM) of seawater (SW) or fish effluent (FE) of the flow-through and batch cultures. Line is linear regression of averages, $R^2 = 0.9628$, $y = 0.4464 + 1.85256 * x$, $p < 0.05$; X- and Y-error bars represent standard deviations of average DIN water and tissue N, respectively.

The N:P ratio calculated from the average water concentrations in the cultures was less than 4 in all four treatments.

4. Discussion

The biomass of *Chondrus crispus* in the four different treatments was able to remove nutrients as shown by the decreases in NH_4^+ , NO_3^- , NO_2 and PO_4^{3-} concentrations, and the seaweed grew by 110 % and 50 % during the 27 days of experiment in the flow-through and batch cultivation systems, respectively.

Nutrient uptake or removal efficiency depends on various physical parameters such as light, temperature, pH and salinity. The water temperature in the flow-through systems remained fairly constant during the day because of the high flow rate through the fish culture and the algal tanks. In contrast, the water temperature in the batch cultures varied with ambient temperature and these variations could have an effect on the fitness of the algae. However, these temperature changes are comparable to those that *C. crispus* may experience in the intertidal zone with rapid temperature change of 10–20 °C in a single immersion-emersion cycle (Kübler and Davison, 1993). The

water temperatures found to be optimal for growth of *C. crispus* are in the range 14–20°C (Bird et al., 1979; Simpson and Shacklock, 1979; Juanes and McLachlan, 1992), and are comparable with the temperatures in this experiment, although Amat and Braud (1990) found maximum uptake of NH_4^+ at a water temperature of 11°C. The pH remained at 8.8 ± 0.2 in all four culture tanks during the experiment and this was probably similar to that in natural conditions, since the seawater pumped in for the experiment was taken from the same area as the natural habitat. However, this pH is higher than that found by Braud and Amat (1996) to give the greatest production for *C. crispus* (pH 8.0–8.2). The salinity in this experiment (30–33 ‰) was also similar to that in the habitat from which the algal material was collected, and this salinity was also found by Neish et al. (1977) to give the highest SGR (31 ‰), although Bird et al. (1979) found optimal growth across a wider range of 10–40 ‰.

Ammonium in fish effluent originates mainly from uneaten food pellets, fish faeces and excretion over the fish's gills as NH_3 . As expected, there was a greater concentration of NH_4^+ in the fish effluent than in the seawater. In addition, most macroalgal species grow better with NH_4^+ as a N-source taken up by diffusion, and the presence of this source inhibits the uptake of other N-sources, although NO_3^- and NO_2^- can be assimilated and actively taken up by macroalgae in the absence of NH_4^+ (Hanisak et al., 1990; Krom et al., 1995; Neori, 1996; Harrison and Hurd, 2001). Indeed, *C. crispus* grows equally well on NO_3^- or NH_4^+ (Neish et al., 1977; Bidwell et al., 1985).

Comparing studies of integrated aquaculture is difficult since different species of seaweed have been used, with different physiological requirements, and there are also differences in stocking densities, nutrient concentrations and flow regimes. The balance of these factors can be adjusted in line with the main purpose of the biofilter, which may be either nutrient removal or biomass production. Low water flow increases removal efficiency, but decreases nutrient availability and thereby decreases the growth of the seaweed. In contrast, high water flow enhances nutrient availability and increases seaweed production, but the nutrient removal efficiency is lower (Subandar et al., 1993; Chopin et al., 2001).

The concentration of nutrients in the fish effluent was low throughout this experiment (total N of 0.07 mg L^{-1} ; total P of 0.11 mg L^{-1}), because the high flow rate through the fish tanks meant that the residence time was very short, and NH_4^+ was undetectable in fish effluent after macroalgal treatment. These results indicated that

the total removal of NO_3^- (approximately 45 %) and PO_4^{3-} (66 %) in the batch cultures was greater than in the flow-through cultures, possibly because of the longer water retention time. However, nutrient availability was higher in the flow-through cultures, resulting in greater total nutrient uptake and thereby more growth, as indicated by the greater final biomass in these cultures. Another study, in which *C. crispus* was integrated with fish production, found the total ammonia nitrogen removal efficiency (NH_4^+ -RE) to be 41 % (Matos et al., 2006) and NH_4^+ -RE from shrimp or fish production has also been investigated using other algal species as biofilters, such as *Porphyra* spp. (70–100 %, laboratory conditions; Carmona et al., 2006), *Porphyra yezoensis* (50–94 %, field conditions; He et al., 2008), *Gracilaria edulis* (97 %, laboratory; Jones et al., 2001), *Codium fragile* (up to 100 %, laboratory batch culture; Kang et al., 2008), *Ulva lactuca* (74–85 %, tank cultivation; Neori, 1996) and *Kappaphycus alvarezii* (71 %, indoor tank cultivation; Hayashi et al., 2008). The removal efficiency for PO_4^{3-} was 36–50 % in the flow-through cultures in this experiment, which compares favourably with other experiments in which *Kappaphycus alvarezii* (27 %) and *Ulva reticulata* (33 %) have been used as biofilters in fish effluents (Msuya et al., 2006; Hayashi et al., 2008).

The specific growth rate (SGR) of *C. crispus* of 0.03 d^{-1} for the flow-through cultures in this experiment was comparable with growth rates of $0.02\text{--}0.04 \text{ d}^{-1}$ recorded on the Atlantic coast of Canada (Chopin et al., 1999) and of 0.03 d^{-1} in greenhouse experiments (Neish et al., 1977). However, our result was less than obtained with the same species in laboratory with different illumination, salinity and temperature conditions (Bird et al., 1979), and similar rates ($0.06\text{--}0.07 \text{ d}^{-1}$) found for the T4 strain in greenhouse conditions (Simpson and Shacklock, 1979).

The increase in biomass of *C. crispus* during the experiment would have been expected to result in an increased nutrient demand and, therefore, an increased rate of nutrient removal. However, such increased nutrient removal was not seen, probably because of low nutrient concentrations in both the fish effluent and the seawater. A lack of nutrients is well known to result in a colour change in the thalli of red algae. Chopin and Wagey (1999) found that, when *C. crispus* was cultured without PO_4^{3-} addition, the thalli changed colour from dark red to light red or green, and eventually fragments broke off from the thalli. In addition, pigments such as chlorophyll and phycobilins can act as N storage compounds and their degradation during N-limitation (high C:N ratio) has been described for macro- and microalgal species such as

C. crispus, *Gracilaria tikvahiae*, *G. foliifera*, *Porphyra yezoensis* and *Rhodomonas* sp. (Lapointe and Ryther, 1979; Bird et al., 1982; Lapointe and Duke, 1984; Chopin et al., 1995; Eriksen and Iversen, 1995; Hafting, 1999). Under high C:N conditions in the medium, the concentration of phycobiliprotein (phycoerythrin) decreases more rapidly than that of chlorophyll *a* and the algae appear bleached (Lapointe and Ryther, 1979; Eriksen and Iversen, 1995). The N:P ratios in the water of less than 4 for all four treatments were lower than the average N:P ratio for macroalgae (30 N:1 P) indicating that growth may have been limited by N in all treatments in the experiment (Harrison and Hurd, 2001), and the bleaching of thalli observed in the batch cultures was most likely because of N- and not P-deficiency. Furthermore, growth rates of macroalgae begin to decrease when the tissue N falls below the critical tissue N (Harrison and Hurd, 2001) and the growth rates of many macroalgae are reduced when the total nitrogen content of the thallus is below 2 % of dry weight (Bird et al., 1982; Hanisak, 1987). The total N in the *C. crispus* thalli at the end of this experiment was higher in the fish effluent flow-through culture (2.5 %N of DW) than in the other treatments, and this level is similar to the 2.6 % found by Amat and Braud (1990) in *C. crispus* grown in different N-NH₄⁺ concentrations. In contrast, the thalli in the three other treatments in our experiment had N-contents below 2 %, supporting N-limitation and even starvation. The thalli in the batch cultures were probably N-deficient during the second day after each water change because the rate of removal decreased (Table 1).

The S contents as an indicator of carrageenan content showed a large variation in thalli from the four different treatments at the end of this experiment.

If maintained for a full year, the production of *C. crispus* in this fish effluent culture would amount to 14 kg DW m⁻³ year⁻¹, and this would be comparable with the results of two other investigations in which this species was grown in fish effluent (extrapolated summer production, 17 kg DW m⁻³ year⁻¹; Matos et al., 2006) or in nutrient enriched seawater (20 kg DW m⁻³ year⁻¹, converted from area to volume; Bidwell et al., 1985).

5. Conclusion

This experiment showed that *Chondrus crispus* could be grown successfully in dilute fish effluent for 27 days in a simple setup in outdoor containers and that the nutrients NH_4^+ , NO_3^- , NO_2^- and PO_4^{3-} were effectively taken up by the seaweed, thus showing the potential to reduce environmental impacts of the fish production. Further refinement of the system should take account of the main purpose of a specific integrated aquaculture scheme, which may be, for example, removing nutrients or maximising the value of marketable seaweed. Greater removal of nutrients from fish effluent could be achieved by choosing species such as *Ulva* and *Gracilaria* with SGR up to 0.18 and 0.09 d^{-1} , respectively (Neori et al., 1991; Haglund and Pedersen, 1993). However, *C. crispus* is a good candidate as a bioremediator species because of its added value as a source of carrageenan. Nevertheless, further investigation is needed to establish its capacity as a biofilter on a larger scale, and to see if this nutrient conversion is profitable or should be an environmental cost in aquaculture. Future regulations could be led by tax incentives resulting in “polluter pays” fees which make nutrient biofiltration a necessity (Neori et. al., 2007). In Denmark, research is in progress on plant lagoons to act as biofilters to reduce nutrients, increase recirculation and thereby decrease the environmental impact of freshwater fish production in on-shore tanks (Dambrugsudvalget, 2002). If successful, such lagoons will probably become mandatory in new or expanded fish farms, in order to qualify for licences. Further research on extractive organisms (filter feeders and seaweeds) integrated with marine fish production will improve the bioremediation of on-shore and off-shore marine fish farms, and could also be required to make fish production more environmentally friendly, as the best technology should always be applied.

Acknowledgement

This project was partly financed by the EU, Marie Curie program (contract number: HPMT-CT-2001-00268) and partly as an Industrial-PhD project (Grant no: 58812) by DHI and the Danish Ministry of Science, Technology and Innovation.

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