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# Application of polychaetes in (de)coupled integrated aquaculture: an approach for fish waste bioremediation

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ABSTRACT: Development of benthic components within integrated multi-trophic aquaculture (IMTA) systems warrants more attention, and the development of polychaetes as an extractive component in IMTA systems is ongoing. This study estimates the bioremediation potential of *Capitella* sp. and *Ophryotrocha craigsmithi* for coupled and decoupled salmon-driven IMTA. In coupled IMTA, polychaetes receive fresh faeces, while in decoupled IMTA, preservation of faeces is applied. Respiration and ammonia excretion rates were measured for polychaetes fed fresh, oven-dried or acidified salmon faeces, and combined with nutrients incorporated into tissue growth, to estimate nutrient requirements. Nutrient requirements were subsequently used to evaluate bioremediation potential. Metabolic rates were highest for *O. craigsmithi* and contributed notably to their overall nutrient requirement (20–30%). For the 2 polychaete species, nutrient requirements ranged from 5 to 26 mg C and from 2 to 6 mg N $g^{-1}$ AFDW $d^{-1}$. These requirements were comparable with or higher than other polychaete species, highlighting the potential for fish waste bioremediation by *Capitella* sp. and *O. craigsmithi*. Preserved diets reduced bioremediation potential 1.5 and 3–5 times for, respectively, *Capitella* sp. and *O. craigsmithi*. Assuming that polychaetes are efficient fish-faeces convertors, the bioremediation potential indicates that benthic cultivation units containing 65 000–95 000 ind. $m^{-2}$ of *Capitella* sp. or 36 000–194 000 ind. $m^{-2}$ of *O. craigsmithi* can convert the daily organic waste flux deposited below an average salmon farm. These densities were within ranges reported for wild populations, indicating that, based on the bioremediation potential, development of benthic IMTA with these 2 polychaete species seems realistic and efficient for waste conversion.

KEY WORDS: Metabolic processes · *Ophryotrocha craigsmithi* · *Capitella* sp. · Benthic IMTA · Integrated multi-trophic aquaculture · Deposit feeders · Sustainability

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## 1. INTRODUCTION

Sustainability issues in aquaculture have fuelled interest in integrated multi-trophic aquaculture (IMTA) systems. In IMTA systems, fed cultures (e.g. fish) are coupled with extractive cultures in such a way that waste resulting from the fed cultures (i.e. uneaten feed, faeces and metabolic excreta) serve as a nutrient source for algae or invertebrates. Due to the potential to reduce aquaculture waste while generating a valuable crop, IMTA is considered to be sustainable (Chopin 2015, Hughes & Black 2016). The concept of IMTA has developed over the years (Chopin 2013, Hughes & Black 2016), and alternative concepts have been presented, including coupled and decoupled integrated systems (Goddek et al. 2016, Jansen et al. 2019). While coupled IMTA refers to the more conventional approach whereby extractive species are integrated in proximity of the fed species, in decoupled IMTA, the different compartments (i.e. fed species and extractive species) are integrated as separate functional units. Spatial connection between the cultivation units is not required in decoupled integrated aquaculture (Goddek et al. 2016).

One of the ecological concerns of finfish aquaculture is the impact of solid wastes on the benthic ecosystem (Taranger et al. 2015, Quiñones et al. 2019). Nonetheless, research on benthic species in IMTA has received less attention than species in the water column (Granada et al. 2016, Filgueira et al. 2017, Jansen et al. 2019, Strand et al. 2019). Deposit-feeding polychaetes have been suggested as potential candidates for benthic IMTA (e.g. Bischoff et al. 2009, Bergström et al. 2015, Gómez et al. 2019, Jansen et al. 2019) and several studies have quantified their bioremediation potential, reporting removal efficiencies ranging from 20 to 85% for organic matter (OM), 65 to 91% for organic C, and 31 to 91% for organic N (Honda & Kikuchi 2002, Palmer 2010, Fang et al. 2016a, Marques et al. 2017, Pajand et al. 2017, Gómez et al. 2019). In the field, it has been demonstrated that the opportunistic polychaete species of the genus *Capitella* Blainville, 1828, which thrives under eutrophic conditions, is able to significantly reduce OM in sediments underneath cage farms and in pond systems (Tsutsumi et al. 2002, Kinoshita et al. 2008). These studies highlight the potential of polychaetes as extractive species in IMTA systems.

Underneath salmon farms, large densities of opportunistic polychaete species are naturally present (Kutti et al. 2007b, Wiklund et al. 2009b, Salvo et al. 2015b). Among these opportunistic species, *Capitella* sp. and *Ophryotrocha craigsmithi* Wiklund, Glover & Dahlgren, 2009 (see Wiklund et al. 2009a) have been identified (Kutti et al. 2007b, Bannister et al. 2014, Valdemarsen et al. 2015). In a recent study, Nederlof et al. (2019) showed that these species grow well on salmon faeces in laboratory trials, and highlighted that based on their nutritive quality, both species seem interesting as a high-quality marine ingredient for aquatic diets. Their ability to convert salmon faeces into valuable biomass shows that *Capitella* sp. and *O. craigsmithi* have potential as extractive species for cultivation in connection with Atlantic salmon *Salmo salar*. In addition, development of benthic cultivation methods for these polychaete species is ongoing (Jansen et al. 2019). Apart from increasing the productivity of a production system, the IMTA approach also strives to reduce wastes produced by fed species via extractive species (Chopin 2013, Hughes & Black 2016). To fully understand the potential of *Capitella* sp. and *O. craigsmithi* as an extractive component in IMTA systems, their bioremediation potential should thus be studied.

Since the grow-out phase of salmon aquaculture is predominantly open-water cage culture, environmental concerns motivate an exploration of alternative production systems, such as enclosed or semi-enclosed sea cages where waste collection could be possible (Lekang et al. 2016). While deposit feeders cultivated underneath open-water cages (i.e. coupled integrated aquaculture) can directly feed on the fresh solid waste, (semi-)enclosed systems make it possible to collect and upgrade solid wastes before use (e.g. decoupled integrated aquaculture). In decoupled IMTA systems, preservation of the collected organic wastes is recommended, as fish waste degrades quickly (Beristain 2005). In the current study, 2 preservation techniques were chosen, drying and acidification. These methods are relatively fast and easy to apply, which makes commercial application more feasible. Both preservation methods may inactivate microbial activity and thereby reduce decomposition rates (Luckstadt 2008, Betoret et al. 2016). However, microbes may also contribute to the diets of *O. craigsmithi* and *Capitella* sp. (Fauchald & Jumars 1979, Findlay & Tenore 1982, Salvo et al. 2015a). Further, compositional changes of solid waste due to preservation potentially affects the nutritional value for the polychaetes, which is most likely to occur during preservation by acidification (Hardy et al. 1984, Özyurt et al. 2016).

The main objective of the present study was to estimate the bioremediation potential of *Capitella* sp. and *O. craigsmithi* fed fresh and preserved salmon

faeces in order to evaluate their application in coupled and decoupled IMTA systems. Respiration and ammonia excretion rates measured in this study, combined with nutrients incorporated in tissue growth (based on data from Nederlof et al. 2019), were used as a proxy to estimate the nutrient requirements (i.e. absorbed nutrients) of the polychaetes. Based on high assimilation efficiencies (AE) reported in previous studies for polychaetes fed aquaculture waste (Honda & Kikuchi 2002, Fang et al. 2016a,b), it can be assumed that the proxies used in this study serve as a valid indication for the nutrient requirements of the polychaetes. Nutrient requirements were subsequently related to deposition of fish waste as a proxy for the bioremediation potential of the 2 polychaete species studied. It should be noted that in this study, bioremediation potential includes recycling of organic waste nutrients by polychaete mineralization (i.e. metabolic processes) and nutrient incorporation in polychaete biomass, but excludes information on consumption and AE, and thus on the amount of waste nutrients that remain in the system.

In addition to the main objective, whether metabolic rates differed when measured either on population or on individual scale was also investigated for both polychaetes. As metabolic rates can be affected by several drivers, the metabolic response of individuals may differ from the response of populations, as a population can be more than the sum of its parts (Hassall 1983). Measurements on a population scale provide information on what can be expected in the field (e.g. cultivation underneath salmon cages), whereas measurements on an individual scale facilitate an understanding of the underlying physiology. *Ophrytorocha* species 'cluster' naturally in dense colonies on organically enriched substrates forming polychaete–mucus complexes (Salvo et al. 2014). *Capitella* species are known to form dense patches in organic enriched sediments (Tsutsumi et al. 2002). It was hypothesized that formation of these polychaete–mucus complexes and dense polychaete patches may influence the maintenance of physiological processes, resulting in differences between measurements on population or individual scale.

## 2. MATERIALS AND METHODS

### 2.1. Species collection

Animals were collected underneath 2 different commercial Atlantic salmon *Salmo salar* farms located in the coastal area of western Norway. One farm was characterized by a soft bottom (125 m depth), where individuals of the genus *Capitella* were collected using a Van Veen grab. In this study, the name '*Capitella* sp.' was used for the species investigated, since the species belonging to the genus *Capitella* occurring in Norwegian waters are morphologically similar (cryptic) and include currently undescribed species. A taxonomic revision of annelids in nutrient-rich habitats (e.g. underneath fish farms) in Norway, including *Capitella* species, is ongoing (T. G. Dahlgren unpubl. data). The substrate below the second salmon farm, where *Ophryotrocha craigsmithi* was collected, was characterized by a hard bottom (140 m depth). For this polychaete, which belongs to a genus of morphologically similar species (Wiklund et al. 2009a), species determination was confirmed using cytochrome *c* oxidase subunit I (COI) barcode data (Hebert et al. 2003). For the collection of *O. craigsmithi*, 3 iron trays (1.2 × 1.2 × 0.1 m, with a perforated base to allow water to pass through), covered with different plastic substrates, were placed underneath the fish farm. The trays were deployed at depths varying between 50 and 150 m, and were left submerged for 3 wk. After 3 wk, the trays were brought to the surface and polychaetes were collected. Both the Van Veen grab and benthic tray samples were carefully washed and polychaetes were collected. Collected polychaetes were immediately placed in an aerated tank containing seawater from 200 m depth, ensuring comparable salinity and water quality to their natural conditions. Then the polychaetes were transported to the experimental facilities of Austevoll Research Station (Institute of Marine Research, Norway), where the experiments were conducted.

### 2.2. C and N mass balance

Nutrient requirements (C and N) were estimated using a mass balance approach based on metabolic processes (measured as respiration and excretion) and nutrients incorporated into tissue growth. Mass balances were calculated for polychaetes fed fresh and preserved salmon faeces. Metabolic processes were measured and the experimental procedure is described below. Nutrients incorporated into tissue growth were calculated based on growth and tissue content data published in Nederlof et al. (2019), which includes a detailed description of how growth and tissue content data were obtained. It should be noted that the experiment described in the present study and the experiment described in Nederlof et al.

(2019) were run in parallel, under comparable conditions and with the same dietary treatments, namely fresh, acidified and dried salmon faeces fed to the 2 polychaete species.

#### 2.2.1. Diet preparation and treatments

*Capitella* sp. and *O. craigsmithi* were fed 3 different diets: fresh, acid-preserved and oven-dried faeces. To prepare the diets, faeces were collected twice a week by stripping salmon (individual weight: ca. 2–4 kg) kept at the sea cage facility of Austevoll Research Station. The collected faeces were directly centrifuged (6300 × *g* for 3 min; Eppendorf 5810R), and liquid was carefully removed. This process was repeated twice. The remaining solid fraction was homogenized and used to formulate the experimental diets. For the fresh diet treatment, polychaetes were directly fed with fresh centrifuged faeces. For the acid diet treatment, centrifuged faeces were preserved by the addition of formic acid (80%), creating a pH < 4 (pH: 3.4 ± 0.1), and left for 24 h at room temperature. Before feeding, excessive liquid was carefully removed and the faeces were washed twice with seawater to reduce acidity. For the washing procedure, seawater was added to the acidified faeces, which was then centrifuged (6300 × *g* for 3 min; Eppendorf 5810R), and liquid was removed. For the oven-dried diet treatment, centrifuged faeces were preserved by oven-drying for 48 h at 100°C, which is assumed to kill the majority of bacteria present in the faeces. Treatments were started on 3 successive days, as preservation of the diets needed different time spans, i.e. Day 1: fresh diet treatment (direct use), Day 2: acid diet treatment (24 h), and Day 3: oven-dried diet treatment (48 h). In total, polychaetes were fed the experimental diets for 2.5 wk.

Prior to the respiration and excretion measurements, animals were kept in 1 l flow-through holding tanks (flow rate: 28 ± 2 ml $min^{-1}$), placed in the dark (ca. 50–70 ind. $tank^{-1}$). These tanks received filtered (1 µm) seawater (salinity: 34.8 ± 0.1, temperature: 8.7 ± 0.2°C) pumped from 200 m depth. These conditions were comparable to conditions measured underneath the salmon farms where the polychaetes were collected (conductivity, temperature and depth scans, data not shown). For *Capitella* sp., glass marbles (5 mm diameter, VWR Norway) were added to the tanks (~1 cm of the bottom was covered), to mimic natural substrates while providing the opportunity to observe animals during daily check-ups. For *O. craigsmithi*, a pre-combusted (overnight, 550°C) stone (~5 cm width and length) was added to the tanks. The rough surface of the stone was assumed to mimic natural substrates for mucus attachment. Animals were fed the experimental diets for 2.5 wk before respiration and ammonia excretion measurements were started by feeding them, in excess (~1.5 g $chamber^{-1}$ $feeding^{-1}$), fresh or preserved salmon faeces twice a week. Leftover feed was always observed, confirming that feed was provided in excess. During each feeding, feed samples were collected and stored in the freezer (–20°C) before analyses.

#### 2.2.2. Respiration and ammonia excretion measurements

Respiration and excretion were measured after animals were placed in clean tanks receiving filtered (1 µm) seawater and were left for 2 d in order to defecate and empty their guts. The next day animals were transferred to the respiration chambers and measurements on metabolic rates started. Directly after the respiration and ammonia excretion measurements, polychaetes were sampled to determine their average weight (on an ash-free dry weight [AFDW] basis). For polychaetes fed fresh salmon faeces, respiration and excretion were measured at both individual and population scales. For polychaetes fed preserved diets, measurements were only done on a population scale. Respiration chambers consisted of closed chambers filled with filtered (1 µm) seawater (salinity: 34.8 ± 0.1, pH: 7.9 ± 0.1). For *O. craigsmithi*, respiration chambers with a volume of 1 and 17 ml for, respectively, measurements on individual scale (n = 1 ind. $chamber^{-1}$) or what was defined as population scale (n = 10 ind. $chamber^{-1}$) were used. Chamber volume for individuals of *Capitella* sp. was doubled (2 ml), as these chambers included marbles to mimic natural substrates (1 ml of the respiration chamber was filled with marbles). Marbles were also added to the population-scale respiration chamber used for *Capitella* sp. (total volume of 17 ml, 4 ml was filled with marbles; n = 13 ind. $chamber^{-1}$). For individual measurements, average weight was 2.1 ± 1.0 and 1.2 ± 0.6 mg AFDW for *Capitella* sp. and *O. craigsmithi* respectively, and for population measurements, average individual weight was 1.8 ± 0.7 and 1.3 ± 0.3 mg AFDW for *Capitella* sp. and *O. craigsmithi* respectively. Population measurements were made with 8 repetitions per treatment (i.e. diet) per polychaete species, while there were 18 repetitions per species for the individual measurements. However, 3 individuals of *O. craigsmithi* died during

the respiration and excretion measurements done on individual scale and were therefore excluded, resulting in a sample size of 15 for this species. For a summary of the experimental set-up, see Table 1.

Oxygen consumption rates were determined using an optical system as described by Rastrick & Whiteley (2011). A PreSens® 10-channel non-invasive oxygen-analysing system (PreSens® OXY-10) was used for the measurements. As preservation of the diets needed different time spans, treatments (i.e. fresh, acid and dried) were run independently per polychaete species. Each population run consisted of 8 experimental (i.e. replicates) and 1 control chamber (i.e. without polychaetes). Individual measurements were performed on the same day, with 2 runs of 9 experimental and 1 control chamber per polychaete species. Oxygen concentrations were logged every 15 s. Incubations were terminated when $O_2$ concentrations in all chambers had decreased at least 20%. All chambers showed a linear decrease in $pO_2$ throughout the incubation period ($R^2$ = 0.6095–0.9963, min–max), showing that oxygen uptake was not affected by handling stress or by changes in oxygen saturation during the measurement period. Oxygen consumption rates were calculated as the difference in the rate of $pO_2$ change between the experimental chamber and the control chamber, multiplied by the volume of the chambers and the solubility coefficient for oxygen in seawater (adjusted for salinity of 35 and temperature of 9–10°C; Benson & Krause 1980, 1984). To convert oxygen consumption rates to carbon loss, a respiratory quotient of 0.9 was used (Cammen 1985).

Following the oxygen consumption incubations, water from each respiration chamber was sampled, including the control chamber (approximately 1 ml for individual measurements and 10 ml for population measurements). These samples were stored at −20°C, for the determination of ammonia excretion rates. Ammonia excretion rates were calculated as ammonia concentrations (see Section 2.3) measured in the experimental chambers minus ammonia concentrations measured in the control chamber, multiplied by chamber volume. Finally, dry matter and ash of the polychaetes were determined, and oxygen consumption and ammonia excretion rates were standardized to 1 g body weight (AFDW) and per hour. Based on the respiration and excretion measurements, O:N ratios were calculated (mol:mol).

Table 1. Summary of the experimental set-up. AFDW: ash-free dry weight

| | *Capitella* sp. | *Ophryotrocha craigsmithi* |
|---|---|---|
| **Experimental conditions** | | |
| Salinity | 35 | 35 |
| Temperature (°C) | 9–10 | 9–10 |
| pH | 8 | 8 |
| **Metabolic rates** | | |
| Population scale | | |
| Start weight (mg AFDW) | 1.8 ± 0.7 | 1.3 ± 0.3 |
| No. of individuals | 13 | 10 |
| Chamber volume (ml) | 17[a] | 17 |
| Replicates | 8 | 8 |
| Individual scale | | |
| Start weight (mg AFDW) | 2.1 ± 1.0 | 1.2 ± 0.6 |
| No. of individuals | 1 | 1 |
| Chamber volume (ml) | 2[a] | 1 |
| Replicates | 18 | 15[b] |
| **Nutrients incorporated in tissue growth**[c] | | |
| Start weight (mg AFDW) | 1.7 ± 0.1 | 1.9 ± 0.1 |
| No. of individuals | 66 | 50 |
| Chamber volume (ml) | 1000[a] | 1000 |
| Replicates | 4 | 4 |

[a]Marbles were added to mimic natural substrates
[b]3 individuals died during the measurements and were excluded from the experiment
[c]Published in Nederlof et al. (2019)

### 2.2.3. Nutrients incorporated in tissue growth

Growth and tissue content (C and N) data reported in Nederlof et al. (2019) for *Capitella* sp. and *O. craigsmithi* fed fresh and preserved salmon faeces were used to calculate the amount of nutrients incorporated in tissue growth. Increase in tissue C and N were first calculated per individual polychaete by the difference in body content (mg C or N ind.$^{-1}$) between the start and end of the experiment. Subsequently these results were standardized for time (per day) and polychaete weight (1 g AFDW). For the latter, geometric mean body weight (mg AFDW) of the polychaetes during the growth period was calculated using the following formula:

$$\text{Geometric mean body weight} = \sqrt{(W_f \times W_i)} \quad (1)$$

where $W_f$ is the average individual final weight (mg AFDW) and $W_i$ is the average individual initial weight (mg AFDW).

## 2.3. Analytical analyses

Diet and polychaete samples were freeze-dried. Diet samples were then ground using a bullet mill.

Diet and polychaetes samples were analysed for dry matter (freeze-dried) and ash (550°C, 6 h). Diet samples were also analysed for C and N content. This was done by combusting the samples with an elemental analyser (Flash 2000, Thermo Fisher) at 1020°C, in the present of oxygen, to convert C and N to $CO_2$ and $NO_x$, respectively. Thereafter, $NO_x$ was reduced to $N_2$ in a reduction column. Nitrogen content was multiplied by 6.25 to estimate crude protein content in the diets. Sampled material was not enough for fat analyses. Nevertheless, diet samples were collected in parallel with samples collected by Nederlof et al. (2019), and therefore diets are assumed to be comparable between the 2 studies. Nederlof et al. (2019) reported fatty acid contents of the diets.

Water samples taken to determine ammonia excretion rates were thawed to room temperature and analysed using the phenol blue method described by Solórzano (1969). Briefly, 8 µl of a phenol alcohol solution (10 g phenol in 100 ml 95% ethyl alcohol), 8 µl of a 0.5% sodium nitroferricyanide solution (1 g sodium nitroferricyanide in 200 ml deionized water) and 20 µl oxidizing solution (100 g trisodium citrate and 5 g sodium hydroxide in 500 ml deionized water; on the day of the analysis, 100 ml of this solution was mixed with 25 ml sodium hypochlorite) were added to 200 µl of the water sample in a well plate (volume: 300 µl). This was left for 1 h at room temperature, after which absorbance was measured using a spectrophotometer (640 nm, SpectraMax M5 with SoftMax Pro software, Molecular Devices LLC). Standard curves were made using ammonium sulphate (1.5 mg ammonium sulphate in 1 l deionized water).

### 2.4. Statistical analyses

Statistical analyses were performed using the R statistical program (version 3.4.0.; R Development Core Team 2017).

Prior to statistical analysis, residuals of the data were checked for homogeneity of variance and normality using Q–Q plots and Shapiro-Wilk and Levene tests. Student's *t*-test was performed to assess potential differences in respiration and excretion rates between measurements on individual and population scale. One-way ANOVA was used to test differences between diet composition (AFDW, C, N and crude protein). As described by Garland & Adolph (1994), statistical comparison of species should be done with care. Since the aim of the present study was to estimate the bioremediation potential of each polychaete species independently, 1-way ANOVA was used to test, within each polychaete species, the effect of diet on respiration, excretion and O:N ratios. Where assumptions of homogeneity of variance or normality were violated, data were transformed. If after transformation, assumptions of homogeneity of variance and normality were still violated, a non-parametric Kruskal-Wallis test was used. When the ANOVA tests were significant ($p < 0.05$), treatments were compared using Tukey's HSD post hoc multiple comparison tests. Significant results found with the non-parametric test were followed by Mann-Whitney *U*-test with Bonferroni correction.

## 3. RESULTS

### 3.1. Diets

Composition of diets fed to the polychaetes can be found in Table 2. Preservation by acidification affected diet composition, as this resulted in a significantly lower AFDW (Tukey HSD; $p < 0.001$), N (Mann-Whitney *U*; $p < 0.01$) and crude protein (Mann-Whitney *U*; $p < 0.01$) content compared to the other 2 diets, which did not differ. C content was significantly higher in the dried diet compared to the acidified diet (Tukey HSD; $p < 0.05$). Both preserved diets did not significantly differ in C content compared with the fresh salmon faeces (Tukey HSD; $p > 0.05$).

Table 2. Composition of experimental diets fed to polychaetes in the weeks prior to respiration and excretion measurements, i.e. fresh salmon faeces and salmon faeces preserved by acidification (formic acid, pH < 4) or by oven-drying (100°C). Values are mean ± SD (n = 6 samples treatment$^{-1}$, except for AFDW of the fresh treatment, where n = 16 samples). AFDW data were log transformed before statistical analyses; non-parametric test was used for potential differences in N and crude protein content of the diets. $^{a,b}$Means within a row lacking a common superscript letter differ significantly ($p < 0.05$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

| | Fresh | Acid | Dried | Signif. |
|---|---|---|---|---|
| AFDW (g kg$^{-1}$ dry matter) | 790 ± 37$^{a}$ | 700 ± 39$^{b}$ | 782 ± 21$^{a}$ | *** |
| C (g kg$^{-1}$ AFDW) | 437 ± 12$^{a,b}$ | 435 ± 11$^{b}$ | 450 ± 4$^{a}$ | * |
| N (g kg$^{-1}$ AFDW) | 24 ± 3$^{a}$ | 14 ± 2$^{b}$ | 25 ± 3$^{a}$ | ** |
| Crude protein (N × 6.25 g kg$^{-1}$ AFDW) | 149 ± 21$^{a}$ | 89 ± 12$^{b}$ | 157 ± 21$^{a}$ | ** |

Table 3. C and N required for metabolic processes based on respiration and excretion and subsequent O:N ratios of *Capitella* sp. and *Ophryotrocha craigsmithi* fed 3 different diets (fresh, acid-preserved and oven-dried salmon faeces). Values are mean ± SD (n = 8 tanks treatment$^{-1}$). All data of *Capitella* sp. and excretion data and O:N ratios of *O. craigsmithi* were log transformed before statistical analyses. [a,b]Means within a row lacking a common superscript letter differ significantly ($p < 0.05$). *$p < 0.05$, **$p < 0.01$, ns: not significant

| | *Capitella* sp. | | | | *O. craigsmithi* | | | |
|---|---|---|---|---|---|---|---|---|
| | Fresh | Acid | Dried | Signif. | Fresh | Acid | Dried | Signif. |
| Respiration (mg C g$^{-1}$ AFDW d$^{-1}$) | 2.94 ± 1.16 | 2.51 ± 0.73 | 4.92 ± 3.85 | ns | 7.88 ± 3.01 | 6.70 ± 2.82 | 9.36 ± 1.82 | ns |
| Excretion (mg N g$^{-1}$ AFDW d$^{-1}$) | 0.39 ± 0.22 | 0.26 ± 0.10 | 0.59 ± 0.42 | ns | 1.37 ± 0.42$^{b}$ | 2.15 ± 0.41$^{a}$ | 2.04 ± 1.09$^{a,b}$ | * |
| O:N ratio (mol:mol) | 14 ± 14 | 15 ± 10 | 12 ± 4 | ns | 8 ± 4$^{a}$ | 4 ± 2$^{b}$ | 7 ± 2$^{a}$ | ** |

### 3.2. Polychaete respiration and excretion rates

Excretion rate of *Ophryotrocha craigsmithi* was affected by diet, and polychaetes fed the acid-preserved diet had a significantly higher ammonia excretion rate compared to polychaetes fed the fresh diet (Table 3; Tukey HSD; $p < 0.05$). Excretion rate of *O. craigsmithi* fed the oven-dried diet did not significantly differ from *O. craigsmithi* fed the 2 other diets (Table 3; Tukey HSD; $p > 0.05$). A significant diet effect was also found for the O:N ratios measured for *O. craigsmithi* (Table 3; 1-way ANOVA; $p < 0.01$); *O. craigsmithi* fed the acid-preserved diet had a significantly lower O:N ratio compared to *O. craigsmithi* fed the other 2 diets (Table 3; Tukey HSD; $p < 0.05$). The latter did not significantly differ from each other (Table 3; Tukey HSD; $p > 0.05$). Respiration rates of *O. craigsmithi* were not affected by diets (Table 3; 1-way ANOVA; $p > 0.05$). For *Capitella* sp., no diet effect was observed for respiration rates, excretion rates and O:N ratio (Table 3; 1-way ANOVA; $p > 0.05$).

Both polychaete species did not show significant differences when respiration and excretion rates were measured either at population or individual scale (Fig. 1; Student's *t*-test; $p > 0.05$). Overall, for *O. craigsmithi*, higher respiration and excretion rates were observed in comparison to rates measured for *Capitella* sp., while *Capitella* sp. showed higher O:N ratios.

### 3.3. C and N mass balances

Fig. 2 shows nutrient requirements of *Capitella* sp. and *O. craigsmithi*, including C and N required for, respectively, respiration and excretion combined with nutrients incorporated in body tissue.

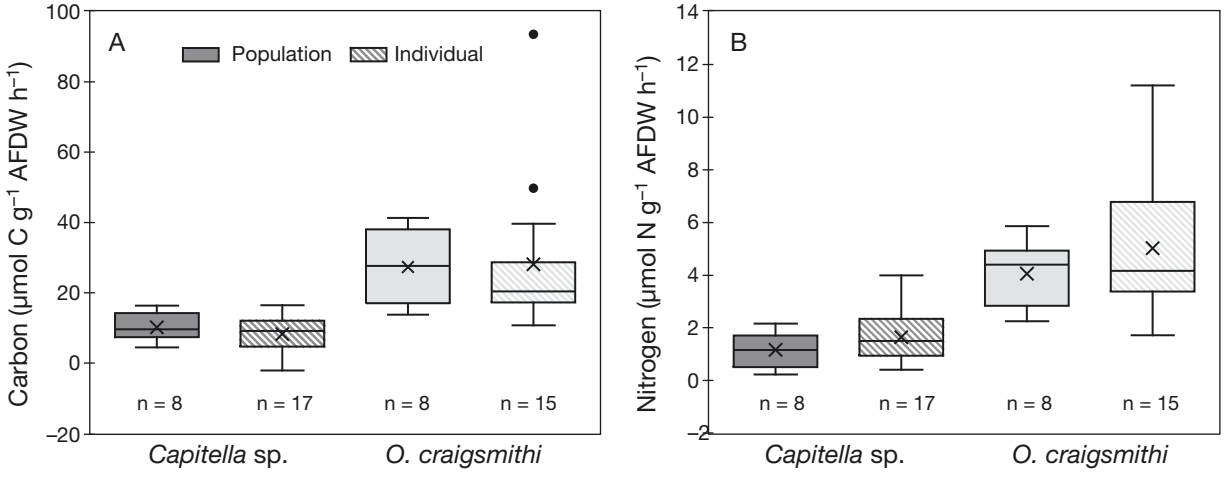


Fig. 1. (A) C and (B) N required for metabolic processes (i.e. respiration and ammonia excretion), measured at population and individual scale for *Capitella* sp. and *Ophryotrocha craigsmithi* fed fresh salmon faeces. C requirement based on respiration measurements; nitrogen requirement based on ammonia excretion measurements. Box: interval between lower and upper quartiles of the distributions; cross: mean value; whiskers: minimum and maximum. Data of *O. craigsmithi* log transformed before statistical analyses. Within each species, no significant differences ($p > 0.05$) were found between measurements at population or individual scale for both C and N requirement

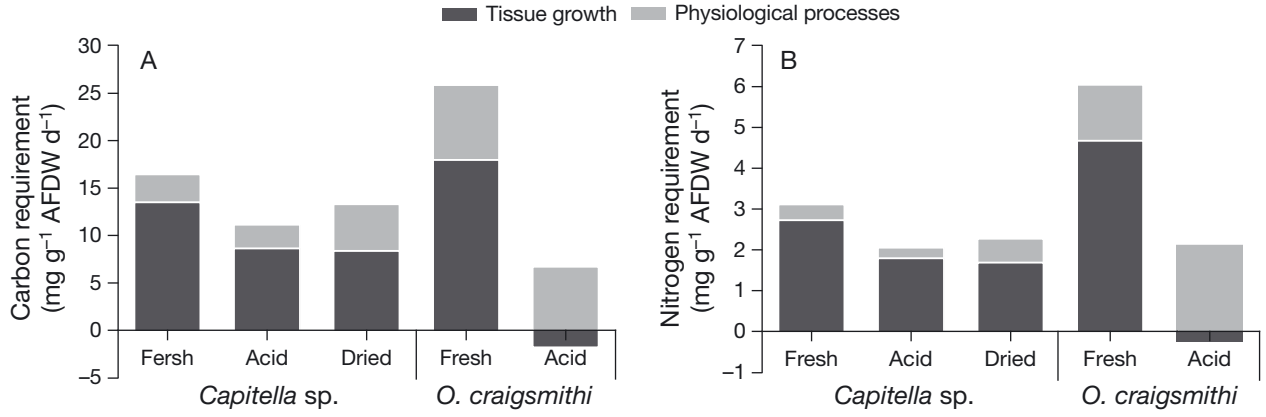


Fig. 2. (A) C and (B) N requirement estimated for *Capitella* sp. and *Ophryotrocha craigsmithi* fed fresh or preserved (acidified or oven-dried) salmon faeces. For nutrients incorporated into tissue growth, growth and tissue content data from Nederlof et al. (2019) were used (see also our Section 2.2.3 for calculation methods). Nutrients required for physiological processes were based on respiration (C) and excretion (N) measurements from the present study (see Section 2.2.2 and Table 3)

For *Capitella* sp., C and N, incorporation rates ranged between 8 and 13 mg C g$^{-1}$ AFDW d$^{-1}$ and between 2 and 3 mg N g$^{-1}$ AFDW d$^{-1}$, with the highest values found for animals fed the fresh diet. Feeding *O. craigsmithi* the acid-preserved diet resulted in negative values for C and N incorporation, due to negative growth rates as reported by Nederlof et al. (2019), while for *O. craigsmithi* fed fresh salmon faeces, the highest C and N incorporation rates were observed (18 and 5 mg g$^{-1}$ AFDW d$^{-1}$ for C and N respectively).

With the exception of *O. craigsmithi* fed the acid diet, for both polychaete species it was observed that growth makes up a significant fraction in the overall C and N requirement (up to 82% for C for *Capitella* sp. on the fresh diet, and up to 87% for N for *Capitella* sp. on the fresh and acid diets). Overall, C requirement ranged between 11 and 16 mg C g$^{-1}$ AFDW d$^{-1}$ for *Capitella* sp. and between 5 and 26 mg C g$^{-1}$ AFDW d$^{-1}$ for *O. craigsmithi*. N requirement ranged between 2 and 3 mg N g$^{-1}$ AFDW d$^{-1}$ for *Capitella* sp. and between 2 and 6 mg N g$^{-1}$ AFDW d$^{-1}$ for *O. craigsmithi*.

## 4. DISCUSSION

This study estimated the bioremediation potential of *Capitella* sp. and *Ophryotrocha craigsmithi* for a salmon–polychaete integrated system. Metabolic rates and the amount of nutrients incorporated in biomass differed between the diets fed, which has consequences for the bioremediation potential in coupled and decoupled IMTA systems.

### 4.1. Polychaete metabolism

#### 4.1.1. Metabolic activity of *Capitella* sp. and *O. craigsmithi*

A 6 times lower respiration rate was measured in this study for *O. craigsmithi* (26–36 µmol $O_2$ g$^{-1}$ AFDW h$^{-1}$) compared to rates reported for *O. labronica* (Rodríguez-Romero et al. 2016). Although factors such as differences in species and experimental set-up should not be excluded, the higher respiration rate observed by Rodríguez-Romero et al. (2016) may be attributed to the higher incubation temperature ($Q_{10}$ rule; e.g. Hochachka 1991), i.e. 20°C in Rodríguez-Romero et al. (2016) versus 9–10°C in the current study. Both respiration and ammonia excretion rates (4–6 µmol N g$^{-1}$ AFDW h$^{-1}$) of *O. craigsmithi* were in line with rates measured during a pilot study for the same species and under comparable environmental conditions (48 µmol $O_2$ g$^{-1}$ AFDW h$^{-1}$ and 7 µmol N g$^{-1}$ AFDW h$^{-1}$) (Brennan 2018), while a twice as high respiration rate was observed in an earlier pilot study for *Ophryotrocha* spp. (Eikje 2013).

Even though, based on incubation temperatures, higher rates were expected, both Chareonpanich et al. (1994) (20°C) and Gillam (2016) (15–20°C) reported, for *Capitella* sp. and *C. teleta* respectively, respiration rates which were in line with the rates measured in our study. Other studies reported higher respiration rates (Brennan 2018, 11°C; Gamenick et al. 1998, 18°C; Linke-Gamenick et al. 2000, 18°C), but lower ammonia excretion rates (Brennan 2018, 11°C; Gardner et al. 1993, 20–25°C) for *Capitella* spp. These results may suggest that metabolic rates are highly

plastic, as was shown for other ectotherms collected from sites different in thermal histories (e.g. latitudes) (Sokolova & Pörtner 2003, Whiteley et al. 2011). Other factors could also play a role in the differences in metabolic rates reported — size differences for example, since animals in both Brennan (2018) and Gardner et al. (1993) had a higher average individual weight than animals used in our study, suggesting that in those studies, the animals were larger. Furthermore, in the different studies, animals might have differed in their physiological status. Metabolic rates are highly influenced by the physiological status of an individual, and changes in O:N ratios can for example provide information on the reproductive status of an animal (Barber & Blake 1985). O:N ratios were measured, but not over time, and conclusions on the reproductive status based on a single time moment can be risky (Jansen et al. 2012). Respiration and excretion measurements were done with individuals from the same batch as used in the experiment in Nederlof et al. (2019), where reproduction in the tanks with *Capitella* sp. was observed, and it is therefore likely that in the current study, metabolic rates of *Capitella* sp. were influenced by their reproductive status. In both our study species, the O:N ratios fall within the ranges indicative for a protein-driven metabolism (3–16) (Mayzaud 1973), but the lower values indicate that the metabolism of *O. craigsmithi* may rely more on proteins compared to *Capitella* sp.

While in the holding tanks, individuals of *O. craigsmithi* clustered together in polychaete–mucus complexes, clustering in dense polychaete patches was not observed for *Capitella* sp. (M. A. J. Nederlof pers. obs.). It was hypothesized that the natural formation of mucus–polychaete complexes observed for *O. craigsmithi* (Salvo et al. 2014) and the formation of dense polychaete patches observed for *Capitella* sp. (Tsutsumi et al. 2002) would affect metabolic rates. No population-specific effects on respiration and excretion rates were observed for both polychaete species, suggesting that measurements obtained on an individual level can directly be scaled to the population level. It should be noted though that the clustering behaviour of *O. craigsmithi* observed in the holding tanks was not seen in the respiration chambers, and it can therefore not be excluded that population-specific effects do play a role.

#### 4.1.2. Effect of diet preservation

Diets used in this study differed in nutrient composition; the oven-dried faeces contained a higher C, N and crude protein content (on an AFDW basis) compared to faeces preserved by acidification, but it was not different from the fresh salmon faeces. In Nederlof et al. (2019), similar diets were used, and a significantly higher total fatty acid content was reported for the fresh and acid diet compared to the oven-dried diet (100°C). In aerobic metabolic processes, macronutrients differ in their degree of oxidation, and therefore respiration is influenced by diet composition (Richardson 1929). Lipid metabolism requires relatively lower oxygen consumption (Richardson 1929), and lower respiration rates for polychaetes fed the fresh and acid-preserved diets can thus be expected compared to the oven-dried diet. Although values were indeed lower, no significant diet effect was found.

No difference in excretion rates and O:N ratios were observed for *Capitella* sp. fed the 3 different diets, while for *O. craigsmithi*, both parameters were affected by diet. The increased ammonia excretion rate of *O. craigsmithi* fed the acid diet compared to the fresh diet, and as a result the reduced O:N ratio, suggests that feeding *O. craigsmithi* with acid-preserved salmon faeces results in a higher protein turnover. This was surprising, since N and crude protein content of the acid diet was significantly lower compared to the other 2 diets, and it remains unclear why *O. craigsmithi* increased ammonia excretion when fed the acid diet. Interestingly, from a bioremediation perspective, the respiration results do suggest that for decoupled systems, preservation of waste by oven-drying would result in a higher C removal, while the excretion results indicate that for N removal, preservation by acidification is preferred.

### 4.2. Bioremediation potential

#### 4.2.1. C and N mass balance

Bioremediation potential can be defined as the relationship between nutrient intake, growth (and reproduction), respiration/excretion and egestion. Several approaches are used to determine this potential, each with their own advantages and disadvantages (Table 4). Which approach is selected is often driven by practical or methodological constraints. In the present study, the initial aim was to define all processes described as approaches II–IV in Table 4. Pilot tests showed, however, that quantification of food intake and assimilation were not accurate due to mucus excreted by the polychaetes — feed and faeces were trapped in this mucus layer, from where it no longer

Table 4. Overview of approaches applied in integrated multi-trophic aquaculture (IMTA) studies to define bioremediation potential of extractive species

| | Approach | Advantage | Disadvantage | References |
|---|---|---|---|---|
| I | Bioremediation is defined as the amount of nutrients incorporated in cultivated or harvested biomass, determined by growth and nutrient content of the extractive species | (1) When extractive species are harvested, this presents the actual amount of nutrients removed from the system<br>(2) Relatively easy method that can be applied in both field and laboratory studies<br>(3) No need to determine the origin (i.e. waste or ambient) of nutrients taken up by the extractive species | (1) Overestimates organic extractive species' maximum cultivation biomass, since waste consumption is not taken into account<br>(2) Underestimates benthic bioremediation potential, since respiration/excretion and bioturbation processes are not taken into account | e.g. Krom et al. (1995), Sanderson et al. (2012), Wang et al. (2012) |
| II | Bioremediation is defined as the amount of nutrients removed due to consumption, determined by food intake rates of the extractive species | (1) Relatively easy method when applied in laboratory studies, with non-mucus-producing extractive species<br>(2) A good method to determine maximum cultivation biomass for organic extractive species | (1) Overestimates bioremediation potential, since egestion is not taken into account<br>(2) A less suitable method for mucus-producing extractive species, when feed, mucus and faeces are hard to separate | e.g. Honda & Kikuchi (2002), Brown et al. (2011) |
| III | Bioremediation is defined as the difference between food intake and egestion, i.e. assimilation, determined by the Conover method, among others | (1) No need for quantitative recovery of extractive species' faeces, and therefore a relatively easy method for non-mucus-producing extractive species<br>(2) Can be applied in both field and laboratory studies | (1) Does not take into account the amount of waste ingested by the extractive species. If food consumption is unknown, this method can only provide qualitative bioremediation, and not quantitative bioremediation<br>(2) A less suitable method for mucus-producing extractive species, when feed, mucus and faeces are hard to separate | e.g. Paltzat et al. (2008), Fang et al. (2016a) |
| IV | Bioremediation is defined by respiration/excretion as a proxy for maintenance of physiological processes | (1) Can be applied to small individuals and mucus-producing extractive species<br>(2) Can be applied in both field and laboratory studies | (1) Underestimates bioremediation potential, since consumption and assimilation are not taken into account<br>(2) Can only be applied when benthic bioremediation is studied | e.g. Honda & Kikuchi (2002) |
| V | Bioremediation is defined by all processes described in approaches II–IV, determined by scope for growth | (1) Provides the most insights into the bioremediation capacity of extractive species | (1) Labour intensive<br>(2) A less suitable method for mucus-producing extractive species, when feed, mucus and faeces are hard to separate<br>(3) Less suitable for field studies | e.g. Jansen et al. (2012), Irisarri et al. (2015), Fang et al. (2016b) |

could be removed. In particular, it was observed (visually) that *O. craigsmithi* 'actively' stored food and faeces in mucus strings. Besides splitting food and faeces, mucus also interfered with OM collection, since filters were easily clogged. Furthermore, the small size of the animals resulted in small faeces fragments, making it difficult to separate polychaete faeces from food leftovers, as is done for larger polychaetes to define assimilation (e.g. Fang et al. 2016a). Instead, bioremediation potential was estimated based on nutrient requirements for growth and metabolic processes. This method excludes information on consumption and AE, overestimating waste turnover capacity. Nevertheless, relatively high AE values have been reported for polychaetes fed aquaculture waste (65–80% for both N and C) (Honda & Kikuchi 2002, Fang et al. 2016a,b). Hence it is assumed that polychaetes are efficient convertors of fish faeces, and that nutrient requirements for growth and metabolic processes provide a good estimate of their bioremediation potential.

Nutrient requirements determined in this study resulted in rates of 16 mg C $g^{-1}$ AFDW $d^{-1}$ and 3 mg

N $g^{-1}$ AFDW $d^{-1}$ for *Capitella* sp. and 26 mg C $g^{-1}$ AFDW $d^{-1}$ and 6 mg N $g^{-1}$ AFDW $d^{-1}$ for *O. craigsmithi* fed fresh faeces. To compare with the literature, these rates were converted to g wet weight (WW), resulting in 3.5 mg C $g^{-1}$ WW $d^{-1}$ and 0.7 mg N $g^{-1}$ WW $d^{-1}$ for *Capitella* sp. and 4 mg C $g^{-1}$ WW $d^{-1}$ and 1 mg N $g^{-1}$ WW $d^{-1}$ for *O. craigsmithi*. Fang et al. (2016b) reported a C and N budget for *Perinereis aibuhitensis* fed defrosted fish faeces. Combining their data on growth and metabolism resulted in requirements of 2 mg C $g^{-1}$ WW $d^{-1}$ and 0.24 mg N $g^{-1}$ WW $d^{-1}$, which is lower than the values obtained in the present study. Honda & Kikuchi (2002) reported a N budget for *P. nuntia vallata* fed flounder faeces, and combining their data on growth and metabolism resulted in a requirement of 0.82 mg N $g^{-1}$ WW $d^{-1}$, which is in line with our study. Interestingly, the C budget for *P. aibuhitensis* presented by Fang et al. (2016b) was dominated by nutrients required for metabolic processes (56–82% of consumed carbon), while in the current study, C incorporated in tissue growth dominated the mass balance. In the N budget of *P. aibuhitensis*, relatively more N was allocated for growth (60–64% of consumed N), compared to excretory N (8–15% of consumed N) (Fang et al. 2016b), which was similar to the current study. Still, especially for *O. craigsmithi* fed fresh faeces, it was observed that nutrients required for metabolic processes made up a notable fraction of the total requirement (20–30%), highlighting the importance of metabolic processes in organic waste reduction by extractive species. Overall, compared to other studies, both *Capitell*a sp. and *O. craigsmithi* show a high potential for waste bioremediation.

### 4.2.2. Farm-scale bioremediation

Organic waste production measured during peak moments of salmon cultivation in a commercial farm in Norway can reach up to 2500 mg organic C $m^{-2}$ $d^{-1}$ (Kutti et al. 2007a), which can negatively impact benthic ecosystems (Kutti et al. 2007a,b, 2008). Based on C requirements determined in the present study, it can be estimated that for 100% removal of the daily organic C waste flux, approximately 65 000 ind. $m^{-2}$ (152 g AFDW $m^{-2}$) of *Capitella* sp. or 37 000 ind. $m^{-2}$ (97 g AFDW $m^{-2}$) of *O. craigsmithi* are required in coupled systems where the polychaetes are provided with fresh faeces (Table 5). These numbers need to increase in decoupled systems, where fish waste is preserved before being fed to the polychaetes (Table 5). It should be noted that the numbers provided in Table 5 are an overestimation, since consumption was not measured. Nevertheless, as mentioned earlier (Section 4.2.1), polychaetes are assumed to be efficient waste convertors (Fang et al. 2016b).

Underneath fish farms, opportunistic polychaete species such as *Capitella* spp. and *Ophryotrocha* spp. can reach high densities (Tsutsumi et al. 2005, Kutti et al. 2007b, Salvo et al. 2015b, Jansen et al. 2019). Tsutsumi et al. (2005) cultivated *Capitella* sp. underneath a fish farm (*Pagrus major* and *Seriola quinqueradiata*) in the Kuusura Bay in Japan and reported population densities of approximately 130 000 ind. $m^{-2}$. Paxton & Davey (2010) estimated that the density of *O. shieldsi* underneath sea cages (*Salmo salar*) at Macquarie Harbour in Tasmania, Australia, reached up to 100 000 ind. $m^{-2}$. Quantitative data on polychaete abundances underneath salmon farms in Norway is limited, as the depth of fjord systems makes it a challenge to measure these abundances (Jansen et al. 2019). However, in the current study, the estimated densities required for 100% waste removal (Table 5) fall within the ranges reported in the literature.

It should be noted that 100% waste removal does not equal 100% waste reduction to the benthic environment, as polychaetes expel faeces as well. Hughes (2016) indicated that there is a need to define IMTA

Table 5. *Capitella* sp. and *Ophryotrocha craigsmithi* biomass and number of individuals required to remove 100 or 20% of the particulate organic carbon (POC) flux to the benthic system of a commercial salmon farm in Norway. Polychaete requirement calculated for coupled (i.e. fresh salmon waste) and decoupled systems where waste preservation is recommended (acid preservation and oven-drying). Deposition rate derived from Kutti et al. (2007a). Carbon requirements are the sum of respiration rates (this study) and tissue growth rates (based on Nederlof et al. 2019). To calculate the number of individuals required, individual geometric mean body weight of 2.35 mg AFDW for *Capitella* sp. and 2.64 mg AFDW for *O. craigsmithi* was used (based on the growth experiment in Nederlof et al. 2019)

| | *Capitella* sp. | | | *O. craigsmithi* | |
|---|---|---|---|---|---|
| | Fresh | Acid | Dried | Fresh | Acid |
| Deposition rate (mg POC $m^{-2}$ $d^{-1}$) | 2500 | 2500 | 2500 | 2500 | 2500 |
| Carbon requirement (mg C $g^{-1}$ AFDW $d^{-1}$) | 16 | 11 | 13 | 26 | 5 |
| **100% POC removal** | | | | | |
| Biomass required (g AFDW $m^{-2}$) | 152 | 224 | 188 | 97 | 511 |
| Individuals required (ind. $m^{-2}$) | 64 643 | 95 353 | 79 935 | 36 664 | 193 632 |
| **20% POC removal** | | | | | |
| Biomass required (g AFDW $m^{-2}$) | 30 | 45 | 38 | 19 | 102 |
| Individuals required (ind. $m^{-2}$) | 12 929 | 19 071 | 15 987 | 7333 | 38 726 |

in terms of environmental performance, raising the question of what percentage of waste should be removed for an effective and sustainable system. In benthic IMTA, the role of microbes in waste mineralization should not be underestimated (Heilskov et al. 2006, Valdemarsen et al. 2012). These microbes can in turn serve as a food source for the polychaetes. The lower bioremediation potential observed for both polychaete species fed the preserved salmon faeces suggests the importance of microbes in their diets, in particular for *O. craigsmithi.* Underneath fish farms, *Ophryotrocha* species commonly form a complex matrix of polychaetes, mucus and chemoautotrophic bacteria (Salvo et al. 2015b), but the role of these matrixes in the bioremediation of fish waste is not yet understood. Next steps in understanding the full bioremediation capacity of benthic IMTA with *Capitella* sp. or *O. craigsmithi* should focus on the interactions that could be encountered at the farm level, like bacteria–polychaete interactions and the aggregation in mucus complexes. It should also be taken into account that opportunistic polychaete species are often characterized by a boom–bust population dynamics, whereby relatively small changes in organic loadings can have large impacts on the population (Ramskov & Forbes 2008). Polychaete cultivation densities aiming for 100% waste removal might therefore be risky. Based on the role of microbes and the risk of population collapses, polychaete cultivation densities required for 20% waste removal are therefore assumed to be more realistic and are also included in Table 5.

While in Nederlof et al. 2019, production potential (i.e. growth and survival) and body composition (i.e. fatty acid and amino acid profiles) of *Capitella* sp. and *O. craigsmithi* fed salmon faeces were evaluated, the present study shows their bioremediation potential for salmon–polychaete integrated systems. Combined, these studies highlight that both *Capitella* sp. and *O. craigsmithi* are interesting species to include in IMTA systems. The next steps should focus on cultivation and harvesting techniques, adjusted to the polychaete species selected. For decoupled integrated systems, this would mean that polychaete cultivation units should be developed, but other waste preservation methods should also be explored, as the bioremediation potential was reduced when polychaetes were fed either acidified or dried salmon faeces. Also for coupled integrated systems, the development of polychaete cultivation methods is still in its infancy, but some steps have been taken. Kinoshita et al. (2008) described, for example, a method whereby individuals of *Capitella* sp. cultivated in a hatchery were introduced under a fish farm. Instead of introducing the polychaetes in coupled integrated systems, there are also opportunities to enhance native polychaete species, as shown by Jansen et al. (2019) for *Ophryotrocha* spp. and other species. This method can be compared with suspended mussel cultivation (Smaal 2002), in which an artificial substrate is provided (ropes in case of mussels, benthic trays in case of polychaetes) on which juveniles can settle and grow and which are eventually harvested. The fast colonization of benthic production systems shown by Jansen et al. (2019) in combination with the high bioremediation potential estimated in this study highlights the opportunity to boost native *Ophryotrocha* species as an extractive component in open water IMTA systems.

### 4.3. Conclusion

This study demonstrates that C requirements range between 11 and 16 mg C g AFDW$^{-1}$ d$^{-1}$ and between 5 and 26 mg C g AFDW$^{-1}$ d$^{-1}$ for *Capitella* sp. and *O. craigsmithi* respectively. N requirements range between 2 and 3 mg N g AFDW$^{-1}$ d$^{-1}$ and between 2 and 6 mg N g AFDW$^{-1}$ d$^{-1}$ for *Capitella* sp. and *O. craigsmithi* respectively. These values were in line with or higher than values reported for other polychaete species, highlighting the potential for fish-waste bioremediation by both *Capitella* sp. and *O. craigsmithi*. The highest values were observed for *O. craigsmithi* fed fresh salmon faeces. Preservation of salmon waste (either by oven-drying or acidification) reduced the bioremediation potential of both species, which could primarily be ascribed to reduced growth. This suggests the importance of polychaete–microbe interactions, and shows that in decoupled integrated systems, higher biomass is required to mitigate fish waste, compared to systems where the polychaetes can be cultivated directly underneath the cages (i.e. coupled IMTA). The polychaete densities required for bioremediation of fish waste lay within the ranges reported for wild populations, and development of benthic cultivation systems boosting these numbers of polychaetes above the natural numbers seems therefore a feasible option.

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