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EDITED BY

Marcel Martinez-Porchas,
Centro de Investigación en Alimentación y
Desarrollo, Consejo Nacional de Ciencia y
Tecnología (CONACYT), Mexico

REVIEWED BY

Jianguang Fang,
Yellow Sea Fisheries Research Institute
(CAFS), China
Jinghui Fang,
Yellow Sea Fisheries Research Institute
(CAFS), China
Stefano Carboni,
Fondazione IMC, Italy
José Lino Vieira De Oliveira Costa,
University of Lisbon, Portugal
Ana Pombo,
Center for Marine and Environmental
Sciences (MARE- IPLeiria), Portugal

*CORRESPONDENCE

Signe Gunborg Bentzrød Svensson
✉ signe.svensson@uib.no

†These authors have contributed
equally to this work and share
third authorship

‡These authors have contributed equally to
this work and share fifth authorship

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Life history traits for *Ophryotrocha craigsmithi* (Wiklund, Glover & Dahlgren, 2009), a candidate species in integrated multitrophic aquaculture

Signe Gunborg Bentzrød Svensson^{1,2,4*}, Tore Strohmeier¹,
Helen Rastrick^{1†}, Antonio Aguera Garcia^{1†}, Erik-Jan Lock^{1*‡},
Harald Sveier^{2*‡} and Henrice M. Jansen³

¹Institute of Marine Research, Bergen, Norway, ²Lerøy Seafood Group, Bergen, Norway, ³Aquaculture
and Fisheries Group, Wageningen University and Research, Wageningen, Netherlands, ⁴Department of
Biological Sciences, Bergen University, Bergen, Norway

Ophryotrocha craigsmithi (Wiklund, Glover & Dahlgren, 2009) has repeatedly been observed in high densities at salmonid fish farms in Norway and has been shown to feed on the organic waste released during fish production. This species has therefore been pointed out as a candidate for the benthic component of integrated multitrophic aquaculture (IMTA). Little is known about the life history traits of *Ophryotrocha* species found at greater depths in temperate waters. Insight into life traits is of key importance in evaluating the bio-mitigation and harvest potential of (benthic) species within IMTA systems. In this study, we aimed to describe key life history traits for *O. craigsmithi*, such as growth, reproductive pattern, and offset of reproduction. The main findings are as follows: (I) *O. craigsmithi* exhibits early sexual maturation and rapid growth, going from 5 to 16 mm in length in 8 weeks; (II) their estimated lifetime is approximately 15 weeks with a generation time of 4 weeks; (III) there are strong indications that they are gonochoric semicontinuous spawners; (IV) the majority of mature eggs ranged from 120 to 150 µm; and (V) the larvae developed into a free-swimming stage after approximately 1 week. To this end, we present a framework for assessing the suitability of deposit-feeding polychaetes as candidate species in IMTA, considering environmental adaptiveness, production potential, as well as carbon and nitrogen mitigation capacity. On the basis of this, *O. craigsmithi* shows a high suitability as an extractive farmed species in benthic IMTA.

KEYWORDS

deposit feeders, polychaete, circular production, mitigation, salmon farming

1 Introduction

In open-water salmonoid farming, a substantial amount of particulate organic waste, consisting of uneaten fish feed and feces, generally settles in the close vicinity of fish farms (Strain and Hargrave, 2005; Broch et al., 2017) and is of ecological concern for the benthic ecosystems (Kutti et al., 2007b; Keeley et al., 2020). For utilizing these waste streams, integrated multitrophic aquaculture (IMTA) offers a strategy for improving system sustainability by taking a circular approach. In IMTA, waste streams from fed species are used to cultivate other extractive species occupying lower trophic levels. This enables diversification and higher utilization of inputs to fish farming while, at the same time, reducing environmental pressure (Troell et al., 2009; Chopin et al., 2012). The capacity of bivalves to mitigate organic particulate waste in open-water IMTA has been questioned (Cranford et al., 2013), and the focus has now shifted toward the benthic part of IMTA. The high amounts of organic waste and its nutritional composition offer an opportunity for utilizing this, today, neglected resource in open-cage farming systems by developing benthic IMTA integrating benthic deposit feeders (Wang et al., 2012; Cranford et al., 2013; Filgueira et al., 2017; Jansen et al., 2019).

A variety of polychaetes are identified as candidates for bioremediation of organic fish waste (Tsutsumi et al., 1990; Fang et al., 2017; Marques et al., 2017). Within the polychaetae genus *Ophryotrocha*, there have been several reports of these species in high densities under commercial fish farms. In Canada, *O. cyclops* is frequently observed in polychaete complexes covering the epibenthos under fish farms (Salvo et al., 2014; Armstrong et al., 2020); and in Tasmania, *O. shieldsi* has been estimated in significant densities under fish farms, up to 100,000 individuals per square meter (Paxton and Davey, 2010). In Norwegian waters, *O. craigsmithi* has recently gained attention, as it has been frequently observed to cover and dominate the benthic fauna under fish farms, especially in areas dominated by hard bottom. This has led to the hypothesis that these have a competitive advantage on organically enriched hard substrates (Hansen et al., 2011; Eikje, 2013; Jansen et al., 2019). The hypothesized advantage of *O. craigsmithi* on hard bottom has led to the idea to develop harvesting structures that offer artificial hard bottom to facilitate the establishment of high *Ophryotrocha* sp. populations (Jansen et al., 2019). The first large-scale harvest structure is under development by a Norwegian salmon company, and the first pilot harvests have been performed on commercial fish farms (unpublished data).

Furthermore, it has been estimated that, a mixed polychaetae community consisting of *Vigtorinella* spp. and *Ophryotrocha* spp. with densities between 8,000 and 71,000 individuals per square meter can be supported under a commercial Norwegian salmon farm (Eikje, 2013). In addition to *Ophryotrochas* sp. high densities at fish farms, they have shown high carbon turnover rates and favorable protein and fatty acid profiles when fed with fish waste (Nederlof et al., 2019; Nederlof et al., 2020). Their nutritional value makes them attractive for the aquaculture feed industry as an ingredient in future feed formulations (Nederlof et al., 2019). The demand for high-quality feed ingredients is increasing as the industry grows, and the need for

these ingredients to be sustainably sourced is crucial for further expansion of the industry (Albrektsen et al., 2022). Combining the traits described above has led to the suggestion of farming *O. craigsmithi* in benthic IMTA systems (Fang et al., 2018; Jansen et al., 2019; Nederlof et al., 2020).

Today, we lack information on population dynamics for *O. craigsmithi*. Information regarding growth and reproduction is a key element in understanding population dynamics, and such life history traits are essential when assessing bioremediation potentials for specific species in IMTA (Ramskov and Forbes, 2008; Galasso et al., 2020). Knowledge on growth and reproduction is further valuable for the establishment of future harvesting strategies of this species in IMTA. *Ophryotrocha* is a well-studied genus with several Mediterranean, shallow, and warm water species held in laboratory cultures (Thornhill et al., 2009). In general, the genus is characterized by opportunistic small worms (< 3 mm in body length) with short life cycles, but the genus shows great variation in their reproductive strategies with species displaying both sequential and simultaneously hermaphroditism as well as gonochoric species (Dahlgren et al., 2001; Kvalø Heggøy et al., 2007). Several *Ophryotrocha* species are described as sequential spawners laying multiple batches of eggs after reaching sexual maturity, and the number of eggs at each spawning and the number of spawning events vary both within and between species (Cassai and Prevedelli, 1999; Thornhill et al., 2009). Norwegian salmon farms are often located over great depths (50–300 m) with a stable temperature of approximately 8°C (Kutti et al., 2007a), representing a very different environment from where the majority of information on *Ophryotrocha* spp. comes from. Life history has been described. Therefore, knowledge regarding the life cycles of *Ophryotrocha* species in temperate waters is limited, and, to the best of our knowledge, only one deep-sea species has successfully been fully reared under laboratory conditions (Mercier et al., 2014). *O. craigsmithi* was first identified and described from a whale carcass on the Swedish west coast in 2009 (Wiklund et al., 2009). Growth rates for this species exist for adult members of the population (Nederlof et al., 2019), but measurements from juvenile to adult stages are lacking. Knowledge regarding reproduction mode and larvae development is also unknown. Because of the limited knowledge for life history traits on *Ophryotrocha* spp. found at greater depths and in temperate waters, it is important to describe these traits for *O. craigsmithi* to enable further assessment of this species bioremediation and harvest potential in IMTA.

The objective of this study is to gain knowledge on key life history traits for *O. craigsmithi*, which are of importance for understanding the population dynamics, nutrient bioremediation, and harvest potential of this species in the context of IMTA farming. By this means, we (I) determined growth rates from juveniles to adults and (II) characterized their reproduction in terms of sexual differentiation, fecundity, and age at onset of reproduction. Finally, we present a framework that evaluates the suitability of deposit feeding polychaetes as extractive components in IMTA. In addition to the key life history traits as described in our study, this framework considers environmental adaptiveness, production and market potential, as well as nutrient mitigation capacity.

2 Materials and methods

2.1 Field collection

Field collection of polychaetes took place at a salmonid fish farm in Hjeltefjorden (60°30'37.0"N 4°57'10.9"E) situated on the west coast of Norway in December 2021. For the collection, we used perforated metal trays adapted from Jansen et al. (2019). These trays were equipped with boxes with lids (18 × 26 × 8 cm, n = 4 boxes per tray). Eight trays were attached to ropes and gently submerged and positioned on the seafloor at depths ranging between 100 and 160 m. Ropes were then attached directly on to the fish cages and left for 4 and 10 weeks. On the day of sampling, the trays were lifted of the sea floor, and lids gently sealed the boxes, reducing the possibility of losing the content of the boxes. Upon retrieval, water quality in the boxes was measured. Temperature of ~8.5°C and salinity of ~34.7 within the boxes confirmed that these had been closed upon retrieval from the seafloor. The boxes were selected on the basis of visual confirmation of successful collection of polychaetes. Boxes were removed from the frames, and, to replace any water that was lost during collection of trays, fresh deep water was added in boxes, kept cool, and transported to the laboratory within 2.5 h after collection.

2.2 Laboratory holding conditions

In the laboratory facility, the boxes were placed into three 40-L flumes provided with a continuous flow (250–400 ml min⁻¹) of unfiltered deep-sea water (8.9°C ± 0.4°C, 34.9–35.1). Flumes were left in the dark. Red light was used during visual inspection of flumes. Given the collection method, the boxes contained complexes consisting of a variety of polychaetes together with organic fish farm waste that had accumulated during the deployment period. Visual observations of boxes showed a clear dominance of *O. craigsmithi*. Throughout this study, polychaetes were fed *ad libitum* with salmon fish pellets (3–7 mm; 40%–47% protein and 24%–32% fat) to simulate fish farm conditions, where food is not assumed to be a limiting factor for growth and reproduction. Before the initiation of the main experiments, polychaetes were allowed to acclimatize for a minimum of 8 weeks. During this time period, pilot studies were performed to establish experimental protocols.

2.3 Length-weight conversions

These polychaetes are mobile and normally contract during disturbance or movement. Thus, these require sedation to obtain actual length measurements. Growth measurements (see Section 2.4) were collected on the basis of non-sedated individuals as multiple measurements in time were collected for the same population and interference was thereby minimized. Length measurements for individuals used for fecundity estimates (see Section 2.5) were defined on the basis of sedated individuals. To allow for conversion between sedated and unsedated polychaete body length, a separate batch of individual polychaetes (n = 158, smaller polychaetes < 7 mm

represented by pooled samples) was sampled from holding flumes and photographed in unsedated state prior to being sedated using isotonic magnesium chloride (75 g of MgCl hexahydrate per liter of purified fresh water) and photographed again. Subsequently, these were dried at 60°C until constant dry weight (~24–48 h) and combusted at 450°C for 6 h to obtain ash-free dry weights (AFDWs; in milligrams). State linear regression was used to define the sedated to unsedated length conversions, and a power function was used to describe the relationship between polychaete length and weight.

Polychaete length is always presented as non-sedated length in millimeters, and conversions by means of linear regression (see Section 2.3) were used when measurements were performed on sedated animals.

2.4 Growth and survival

From holding flumes, a small subset of individuals was selected and measured during an 8-week-long growth trial. Polychaetes were sorted according to total body length measured with the use of millimeter paper from the anterior to last segment posteriorly. Individuals were sorted in three size groups: large = 13.3 ± 0.6 mm, medium = 7.9 ± 0.7 mm, and small = 4.8 ± 0.5 mm (mean ± SD). The smallest size group was set to the given size due to practical limitations for working with smaller individuals. The largest size group represented the largest individuals sampled in the maintained population. To ensure correct species collection, the morphological traits described by Wiklund et al. (2009) were used during sorting. Groups of polychaetes were kept isolated in 8.5-L flow-through chambers (n = 50 individuals per chamber; n = 3 chambers per size group) with a flow rate of 23 ± 0.3 ml min⁻¹ and temperature of 8.3°C ± 0.3°C. Polychaetes were fed in excess with salmon feed (4.5 mm; 43%–46% protein and 25%–28% fat) twice a week with ~105 mg per feeding event. This was to simulate fish farm conditions, where food is not assumed to limit growth of polychaetes. On a weekly basis, images of all chambers were collected using a SLR camera (Canon EOS 600D, EFS 18-55 mm). To obtain a high-quality image of all individuals at once, polychaetes were gently directed toward the bottom of each chamber, and, when normal behavior was resumed, after being disturbed, images were collected. Images were taken with a scale (millimeter paper placed on the bottom of each chamber), and images were later imported, scaled, and analyzed with ImageJ software Java 1.8.0191 (Schneider et al., 2012) to measure polychaete total body length. To obtain survival data, all individuals observed on images were counted on a weekly basis. The method of using images reduced handling of polychaetes to a minimum.

Growth rates were calculated as length-specific growth rates (LSGRs) by the equation given below:

$$LSGR \% \text{ day}^{-1} = \left(\frac{\ln(Lt) - \ln(Li)}{T} \right) * 100$$

where Lt is the total body length (in millimeters) at time of measurement, Li is the initial total body length (in millimeters), and T is the number of days passed since the first measurement.

2.5 Fecundity

Fecundity in this experiment is defined as the number of mature eggs spawned per spawning and individual; hence, we are not including immature eggs to consider potential fecundity or following specific individuals to estimate lifetime fecundity (total number of eggs laid throughout a lifetime). Two separate experiments were performed because isolated individuals did not spawn naturally but were observed to spawn in larger groups. First, the size variation, measured as egg diameter, of naturally spawned eggs was investigated to define a size range for mature eggs. Second, the number of mature eggs was counted and measured after induced spawning by heat-shock treatment, for an estimate of individual fecundity.

2.5.1 Definition of the size for mature eggs

To obtain naturally spawned eggs, polychaetes were sorted out in three size groups ($n = 3$ per group; $n = 20$ per chamber) represented by the sizes of 15.8 ± 0.4 mm (large), 8.1 ± 0.6 mm (medium) and 5.1 ± 0.2 (small) in length (mean \pm SD). Spawning chambers consisted of 0.8-L containers provided with a flow rate of 11 ± 2 ml min^{-1} and temperature of $8.3^\circ\text{C} \pm 0.2^\circ\text{C}$. Every morning, containers were visually inspected for eggs. Spawned eggs were collected and put on ice. Each egg sample was sub-sampled ($n = 3$) for eggs with the aim of collecting a minimum of 10 eggs per sub-sample. Eggs were fixed on a microscopic slide with Metcel (Methyl cellulose, M7027) and photographed (Canon DS126201). Images were analyzed in ImageJ software where images were scaled using a microscale slide. From images, we measured egg diameter and categorized the first stages of egg development (Figure 1). The remaining eggs from each sample were diluted in filtered seawater ($0.2 \mu\text{m}$) and measured by a laser particle counter (PAMAS S4031GO) in triplicates. Every evening, chambers were cleaned from leftover food and eggs before provided with a fresh feed pellet. This trial was done over the course of 7 days.

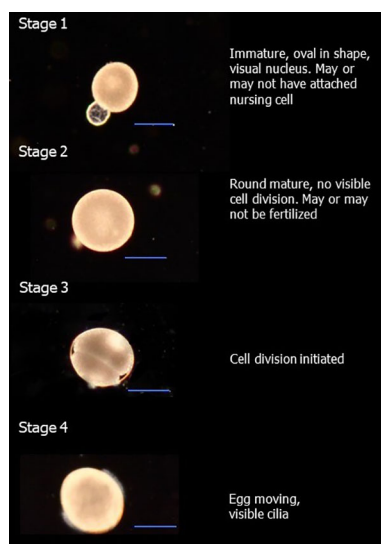


FIGURE 1
Developmental stages in eggs used for categorization during natural spawning's. Scale bars represent $100 \mu\text{m}$.

2.5.2 Individual fecundity

Prior to choosing the heat-shock method, pilot studies were performed and evaluated mechanical opening of individuals, isolation of separate animals/pairs, and heat-shock treatment at different temperature elevations. The final protocol (see below) provided predictable spawning events and comparison of manual counting with PAMAS measurements ensured satisfying precision between the two methods.

Polychaetes were collected from holding tanks on the basis of the total body length, and females were then identified on the basis of coloration and visible eggs through the body wall. Each individual was placed in a separate well in a well plate together with 10 ml of filtered ($0.2 \mu\text{m}$) sea water. The sea water temperature was 3.5°C higher compared with that in the holding-flume conditions. Plates were left in room temperature ($19.1^\circ\text{C} \pm 0.9^\circ\text{C}$) for 2–2.5 h. This time was sufficient for polychaetes to spawn without compromising egg quality, because it was observed that eggs left for more than 3 h could become fragile and easily break during handling. After spawning, polychaetes were gently removed from each well, and eggs were put on ice, whereas polychaetes were placed in a fridge until further handling (2°C). Each egg sample was diluted with 50 ml of filtered ($0.2 \mu\text{m}$) sea water. A magnet stirrer provided gentle stirring, to suspend the eggs, during measurements by the PAMAS, as described in Section 2.5.1. Afterward, each individual was sedated in isotonic MgCl (see Section 2.3), and the total body length was measured. Subsequently, individuals were dissected and checked if spawning had been successful. For smaller individuals where sex is hard to distinguish, dissected polychaetes were checked under microscope at $\times 40$ magnification. On the basis of results derived from Section 2.5.1, counting of eggs started at egg diameter $> 120 \mu\text{m}$.

2.6 Histology

Histology was used to determine the onset of gamete production in *O. craigsmithi*. Polychaetes ($n = 30$) were collected on the basis of their total body length and included the range of 4–16 mm from the stock population. Differentiation between assumed sexes was based on coloration, visible eggs through the body, and the width of the back-part, as it was observed that males appeared to be broader toward the end compared with females (personal observation, see Figure 2). Individuals were sedated with isotonic MgCl solution, measured, and washed in filtered seawater before being fixed in Davidsons fix with 5% acetic acid for 24–48 h. Samples were dehydrated and casted in paraffin (Histowax, 60°C – 63°C) before being sliced ($3 \mu\text{m}$) with a microtome (Thermo Scientific, HM355S), stained with Instant hematoxylin (Thermo Scientific), and photographed with NanoZoomer S60 Digital (HAMAMATSU). Images were analyzed in NDP.view2 U12388-01 (HAMAMATSU).

2.7 Courtship behavior and larvae development

On a few occasions, spawned eggs were collected and isolated in cups or well plates (20–100 ml) before placed in a climate room of $\sim 10^\circ\text{C}$, without aeration. These were checked intermittently to record

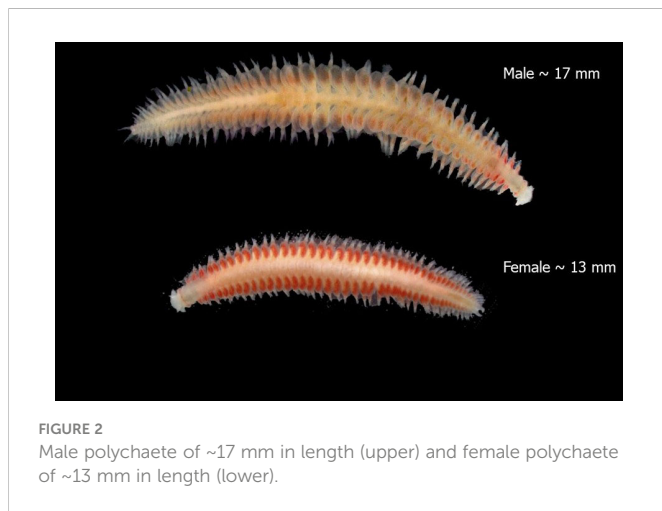


FIGURE 2
Male polychaete of ~17 mm in length (upper) and female polychaete of ~13 mm in length (lower).

new developmental stages. Courtship behavior was observed in the holding tanks on multiple occasions and caught on video.

2.8 Statistics

Statistics were computed using Rstudio (Rstudio, Team 2022). All data are presented as means \pm SD unless stated otherwise. To describe the weight-to-total body length relationship, a non-linear least squares model was used to estimate the power-parameter b and the coefficient a by the equation: $\text{weight} \sim a \times (\text{sedated length (in millimeters)}^b)$. Growth data were used in a von Bertalanffy growth model to estimate the following parameters: the asymptotic length (L_i) and the intrinsic growth rate (rb) of the population by the equation $L \sim L_i - (L_i - L_0) \times \exp(-rb \times (\text{time}))$. von Bertalanffy parameters were used to calculate the approximate age of the polychaetes at the start size of the different size groups using the following von Bertalanffy formulation: $t(\text{time}) = \log((L_i - L_0)/(L_i - L))/rb$, where time is in days since reaching L_0 , L_0 is the mean initial size of the small groups of *O. craigsmithi*, and L is the mean size at measuring event for the other measurements event and groups ($n = 3$) during the growth trial. To investigate the potential correlation between egg size and total polychaete body length, a linear correlation test was used with $p < 0.05$ set as the significance level.

3 Results

3.1 Relationship between polychaetae length and weight

The relationship between unsexed and sexed length of polychaetes is given by the equation $y = 0.6024x + 1.1314$ ($R^2 = 0.85$, $p = 2.2 \times 10^{-16}$ with $x =$ unsexed length (in millimeters) and $y =$ sexed length of polychaete (in millimeters). The parameters provided by the non-linear least squares model were $a = 0.0092$, $p = 0.0236$, and $b = 2.3038$ ($p < 2 \times 10^{-16}$), resulting in the following equation for describing the weight to sexed polychaete length relationship: $\text{AFDW mg} = 0.0092 \times y(\text{mm})^{2.3038}$ ($R^2 = 0.78$).

3.2 Growth and survival

The calibration of the von Bertalanffy growth model gave the intrinsic growth rate of $rb = 0.0237$ mm and asymptotic length of $L_i = 20.588$ mm for *O. craigsmithi*. This further resulted in start age for the medium and large polychaete groups at 9 and 32.5 days, respectively (Figure 3B). The model also showed that the time for a 0.3-mm polychaete to reach the starting size of the small polychaetes used in our experiment was 11 days. Furthermore, they showed an estimated lifetime of ~15 weeks and a generation time of 4 weeks. Growth data revealed that the smallest size group of polychaetes had the highest LSGR of $4.2\% \pm 0.5\%$ day^{-1} (Figure 3D). This group also doubled in length from 4.8 ± 0.5 mm to 11.0 ± 0.3 mm in length in 3 weeks before reaching their close to maximum size of 16 mm after 8 weeks (Figure 3A). The medium-sized polychaetes showed a maximum LSGR of $2.9\% \pm 0.6\%$ day^{-1} during the first week of the experiment, which decreased to $1.3\% \pm 0.2\%$ day^{-1} when polychaetes had reached a size of approximately 16 mm. The largest size group showed a slight increase in length and had a maximum growth rate of $1.7\% \pm 0.1\%$ day^{-1} in the second week of the growth trial. Three weeks into the experiment, the growth decreased slightly for all three size groups. After the second week, the large group growth rates were stabilized, and their survival was down to $49\% \pm 22\%$ after 3 weeks. The large groups continued to steadily decrease in numbers and survival was $23\% \pm 7\%$ by week 6. In the last week of the experiment, only $17\% \pm 4\%$ of the individuals were rearing. Medium- and small-sized groups showed high survival throughout the experiment, and, by the end of the growth trial, the medium group still maintained a survival of $91\% \pm 3\%$. Survival data (Figure 3C) show a variation in the number of polychaetes counted over time for the medium- and small-sized groups; this is explained by the difficulty to distinguish the smallest polychaetes in the beginning of the growth trial.

3.3 Histology

The sampled population consisted of 43% females and 53% males, and 4% of the individuals were not able to be determined. The smallest female sampled, 6 mm in unsexed length, contained oocytes in different stages of maturation. This trend for eggs in different maturation stages was consistent for the females sampled in the total body length range of 6–21 mm (Figure 4). All males longer than 7 mm contained a combination of mature/immature sperm, whereas half of the small males (5–7 mm; $n = 6$) showed only immature sperm. Egg and sperm were never observed in the same individual.

3.3.1 Natural spawning

Eggs collected from natural spawning events were dominated by the sizes between 130 and 140 μm (Figure 5). The development stages (see Section 2.5.1 for definitions of stages) of all eggs, laid by the large group of *O. craigsmithi* ($n = 635$), showed 80% as stage 2 and 17% as stage 3, as determined by microscopy. Eggs within stage 2 displayed a

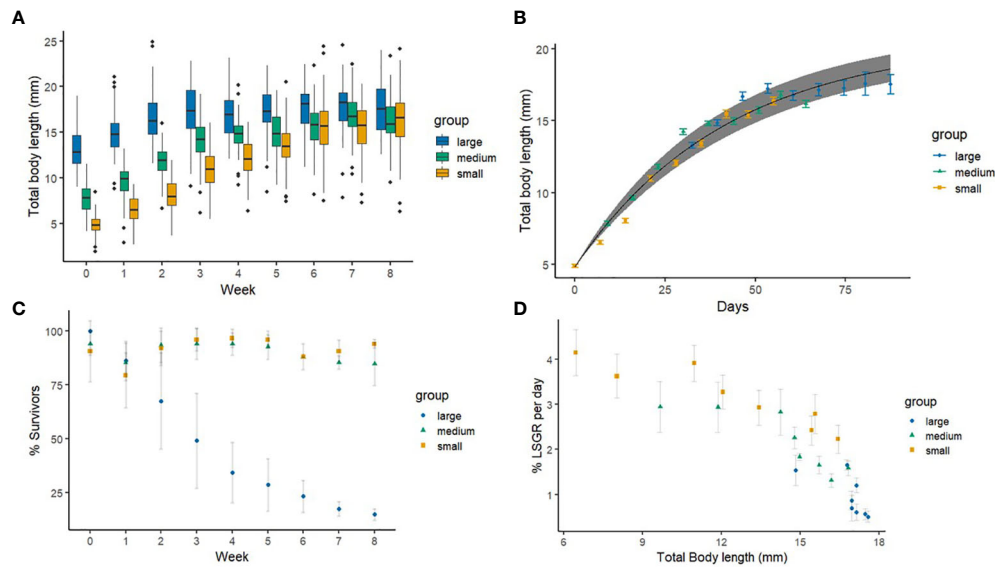


FIGURE 3

(A) Body lengths (in millimeters) for pooled polychaete groups over time with black horizontal line showing the median and vertical lines, illustrating minimum and maximum values. (B) The von Bertalanffy growth curve plotted together with measured data for mean total body length (mean \pm SE) with the line for predicted mean values and 95% confidence interval. Polychaetae groups are plotted according to their calculated age (days). (C) Total survival (%) over time ($n = 3$ per group, mean \pm SD). (D) Length-specific growth rate (LSGR % day⁻¹) ($n = 3$ per group, mean \pm SD). Size groups are separated by shape and color where blue circles = large, green triangles = medium, and yellow squares = small.

large range in egg diameter from 115 to 202 μm . During the collection of naturally spawned eggs, both medium and large groups spawned, but only the large group laid sufficient amounts of eggs for measurements with the laser counter. However, eggs that were collected from the medium-sized groups followed similar trends in the egg size distribution. The eggs at sizes defined as mature ranged from 120 up to >190 μm with a clear peak at size of 140 μm in diameter (Figure 5). Eggs larger than 200 μm in diameter were hypothesized to swell due to change in osmotic pressure or have gone through multiple stages of cell division. Eggs smaller than 120 μm in diameter corresponded to stage 2 (see Figure 1) and were therefore not categorized as mature.

Courtship behavior was frequently observed in the holding tanks. Male and female would attach, and one individual appeared to twist itself around the other. Subsequent vigorous “wiggling” took place before a small white cloud was emitted into the water and settled on

the bottom (see Supplementary Material for video). Pipetting of this material confirmed that there were eggs that had been fertilized, and a couple showing courtship behavior was also included in histology, which confirmed that the couple consisted of male and female.

3.3.2 Individual fecundity

There was an apparent variation in the number of eggs spawned by individuals of a similar length (Figure 6). There was no linear correlation between the total body length and the number of spawned eggs ($p = 0.63$). The majority of polychaetes spawned less than 250 eggs, whereas the maximum number of spawned eggs was 1,063 spawned by an individual of 13.1 mm. Mature eggs were not released by individuals smaller than ~ 6 mm in length, and neither were immature eggs observed during dissections on the same individuals. Seventeen large (> 10 mm in length) individuals also did not spawn

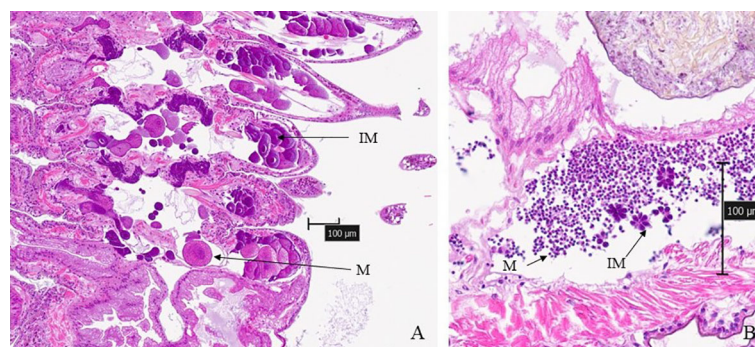


FIGURE 4

Histological section of (A) 21-mm-long female and (B) 25-mm-long male. IM, immature gametes; M, mature/maturing gametes. Scale bar, 100 μm .

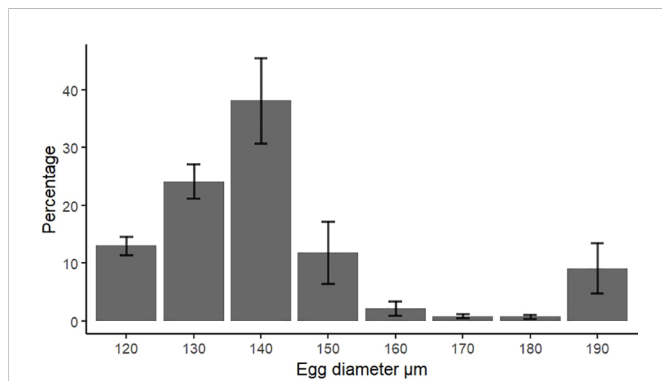


FIGURE 5
Size distribution of mature eggs (mean \pm SD) collected during natural spawning events from the large polychaetes (N = 3 chambers, n = 20 polychaetes per chamber). "Mature" eggs defined by microscopy (Figure 1).

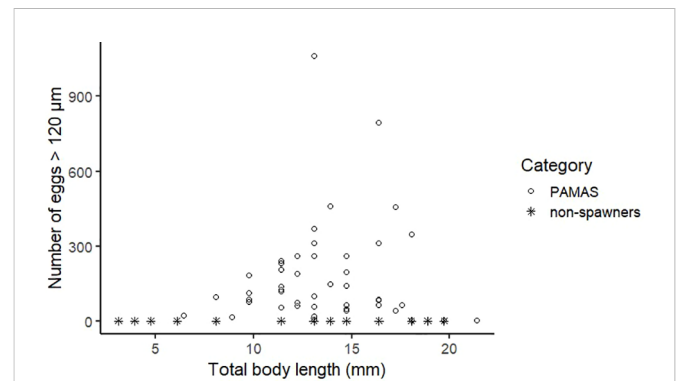


FIGURE 6
Individual fecundity as number of eggs > 120 μ m released per individual after heat-shock treatment (n = 73). Circles represent spawning individuals, and asterisks represent non-spawning individuals (34% of individuals included did not spawn).

after heat-shock treatment, but dissection revealed that these individuals sometimes contained a vast number of immature eggs.

3.4 Larvae development, mucus production, and general behavior

Determination of developmental stages showed that fertilized eggs had started and gone through early cleavage within 6 h after collection (Figure 7). After \sim 15 h, we observed slow circular movements in the eggs. A free-swimming trochophore-like stage appeared after 70 h. This has developed later into a metatrochophore with clearly visible ciliated bands. The first segment appeared after 114 h and grew into three- to four-segment-long free-swimming larvae without chaeta after 1 week. Although there were several feeding attempts of larvae, offering live microalgae and different types of aquarium fish feed, there was no successful rearing of larvae beyond this point. This timeline for development of larvae should be interpreted with care due to few successful replicates.

Colonies of polychaetes formed three-dimensional-like structures with mucus along the edges of the holding tanks. Mucus was frequently sampled to investigate whether mucus were used for the development of larvae; however, larvae was never observed inside mucus. Individuals were very mobile and could quickly (hours to minutes) localize feed introduced to the flumes.

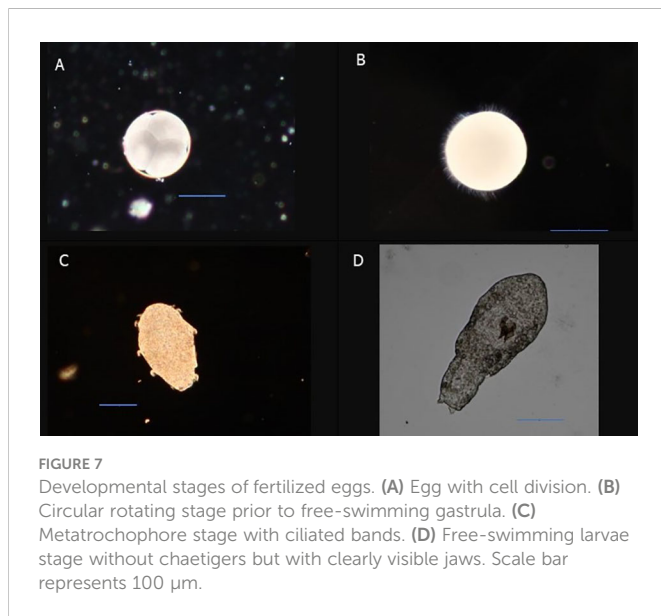
4 Discussion

In this study, we investigate key life history traits necessary for assessing *O. craigsmithi* as a candidate species in IMTA. We find that these polychaetes fed on a diet of fish pellets obtained high growth rates and have a short generation and lifetime. Furthermore, this species is gonochoric and, most likely, a semicontinuous spawner. They have a free-swimming larvae stage that develops after approximately 1 week after fertilization at \sim 10°C. After assessing life history traits, nutrient mitigation, and production potential, the high potential for *O. craigsmithi* as an extractive species in (benthic)

IMTA is strengthened, and more knowledge regarding the population growth and biomass under farms is the next step for understanding their future role in IMTA systems.

4.1 Growth, survival, and lifetime

Small individuals doubled in length in the first 3 weeks of the growth experiment, and, during this time frame, polychaetes went from non-spawning members of the population to spawning adults. The decreased growth measured for the adults coincided with increased mortality. At this stage, polychaetes were estimated to be 50 days old, indicating that this species has a short lifespan. The estimated lifespan for this polychaete is \sim 15 weeks, and the generation time is \sim 4 weeks. Their lifetime and generation time are comparable with those *Ophryotrocha* sp. measured at temperature rates of 15°C and 24°C (Åkesson, 1973; Simonini and Prevedelli, 2003). However, their lifetime is significantly shorter than the approximately 34 weeks observed for a temperate deep-sea *Ophryotrocha* sp. reported by Mercier et al. (2014). The high mortality rate measured for the large groups in the growth experiment was not observed in the two other size groups, even when their estimated age and size were comparable. The reason for this high survival remains unknown, and future growth studies should last until at least a 50% mortality rate is observed to further assess their lifetimes. The growth rates in this study (1.5%–2.9% day⁻¹ for individuals between 12 and 14 mm in size) are comparable with previous results for similar sized individuals of the same species reared on a diet of freshly collected fish feces (Nederlof et al., 2019). These combined results indicate a high capacity for growth when reared on different waste fraction from fish farms. Care should be taken when comparing growth rates between species as the specific growth rate calculations include the starting size and the time interval between measurements, which are generally study and species specific. Nevertheless, these rates can be seen as an indication of the ability of a species to grow on a specific diet in a given environment. Wang et al. (2020) not only demonstrated the high growth rates of juvenile *H. diversicolour* reared in the laboratory with a commercial fish feed diet but also highlighted that this could vary based on the type of diet and the



substrate provided. We assume that optimal growth rates were measured in our study given the high food quality provided in the growth trial. Furthermore, growth rates measured for young adults in our study (8–12 mm in length) were in the same order of magnitude to what has been observed for other polychaete species reared on aquaculture waste such as *H. diversicolor* (Wang et al., 2019) and *Capitella* sp (Nederlof et al., 2019). It can be hypothesized that the decreasing growth observed approximately 15 mm in size is linked to energy allocation to reproduction. For *O. labronica*, such investment into reproductive tissue has been proven to be significant (Cassai and Prevedelli, 1999).

4.2 Reproduction

We further conclude that this is a gonochoric species on the basis of histology and the morphological characteristics distinct for individuals carrying eggs or sperm. The genus is represented by species with different modes of reproduction, but gonochoric is the most common one (Thornhill et al., 2009).

Several observations in our study support that *O. craigsmithi* is a semicontinuous spawner. First, the variation in the number of spawned eggs was large during spawning by individuals of a similar size. For example, for the polychaetes between 13 and 16 mm in length, the number of eggs varied between 0 and >1,000 for a single spawning event, suggesting that several batches of mature eggs could be produced after reaching reproductive age. Despite the large variability across sizes, it seems that the number of released eggs is related to polychaete size or age. A similar pattern has been described for *O. puerilis*, where the number of eggs increased from 30 eggs during first spawning to 300–400 eggs in older individuals (Åkesson, 1967). Second, dissections of spawned polychaetes revealed the presence of remaining, mostly immature, eggs. This, in combination with histology showing that all females (7–20 mm) contained eggs in several stages of maturation, builds on the first argument for semicontinuous spawning. Finally, they appear to have the ability to spawn naturally already at half of their maximum size (4 weeks of age), and, at this point, survival was high. Semicontinuous

spawning is a common trait within *Ophryotrocha* (Åkesson, 1973; Thornhill et al., 2009) and is thought to be an important trait for opportunistic species as a way of quickly responding to changing environments (Tsutsumi, 1987). Given that the number of spawning's per individual remains unknown, the lifetime fecundity could not be determined in our study. To do so, it requires investigation of individuals over time, which was not possible in our study as isolated individuals/couples did not spawn. The resistance to spawn spontaneously in isolation has also been observed for the temperate *O. cosmetandra* (Oug, 1990). It is reasonable to assume that the high nutritional value provided in this study may have maximized the reproductive output in terms of number of eggs. The degree of importance of food quality and its potential influence on fecundity has been demonstrated for *Dinophilus gyrociliatus*, where a high-quality feed resulted in a two- to three-fold higher fecundity (Prevedelli and Simonini, 2000).

The size of mature eggs (120–160 µm) was within ranges as reported in other *Ophryotrocha* spp (Paxton and Akesson, 2010; Mercier et al., 2014). However, the range in size for mature eggs seems to be large. The egg size and number are important parameters, indicating the amount of energy invested in reproduction by the individual that affects the population growth of the species (Simonini and Prevedelli, 2003). Spawning behavior revealed that eggs were released freely into the water before gently sinking to the bottom. This is unusual among *Ophryotrocha* because most species prepare some type of mucus cocoon or loose jelly where they deposit their eggs (Wilson, 1991; Thornhill et al., 2009), showing that this species does not put any effort in brood care. On the other hand, the timeline of larval development (~10°C) converged with patterns observed within *Ophryotrocha* spp. reared at 15°C–25°C (Åkesson, 1973), and the first stages of development were similar to that in *O. shieldsi* collected under a Tasmanian fish farm (Paxton and Davey, 2010). However, their development was faster than what has been reported for the temperate *O. cosmetandra* whose early development at 5°C–8°C took 30–50 days (Oug, 1990), implying that *O. craigsmithi* is highly adapted to rapid colonization and spreads and grows in temperate environments.

4.3 Implications for benthic IMTA

Insight into life history traits is of key importance when evaluating the suitability of a species for IMTA. To fully assess their potential, multiple aspects need to be considered. We therefore present a framework (Table 1), identifying the requirements for benthic polychaetes to be in IMTA. The following factors are considered: environmental and biological suitability as well as nutrient mitigation capacity and market potential.

Several polychaete species show potential as candidates in IMTA, all with their pros and cons (Table 1). The major challenges for successful incorporation of polychaetes in IMTA are often connected to controlling reproduction to have continuous access to polychaetes and to optimize the trade-off between biomass increase and rearing densities (Nesto et al., 2012; Palmer et al., 2014; Pombo et al., 2020; Wang et al., 2020). *O. craigsmithi* is not only among the smallest polychaetae candidate in our comparison between species in IMTA settings but also one of those reported to reach some of the highest densities under fish farms (Paxton and Davey, 2010; Eikje, 2013; Hamoutene et al., 2013). Furthermore, *O. craigsmithi* shows high

carbon and nitrogen mitigation potential and high growth rates (Nederlof et al., 2020). Short generation time accompanied with semicontinuous reproduction would result in high population growth and allow for multiple harvests during a single fish production cycle that could increase the total yield for harvested polychaetes. *Capitella* sp. has been successfully seeded in organically enriched sediment under fish farms and has reached densities as high as 134,000 individuals per square meter (Kinoshita et al., 2008), showing its potential for being applied at sites situated over soft bottom substrate. *Pereneris aibuthiensis* has also shown promising nutrient mitigation potential when combining their organic removal efficiency and required densities for successful bio-mitigation under fish farms (Fang et al., 2017). Furthermore, *H. diversicolor* is a promising candidate for closed systems. Marques et al. (2017) reported a 70% reduction in organic material when *H. diversicolor* was integrated in sand filter systems and provided with aquaculture waste. Both *H. diversicolor* and *Pereneris* sp. are generally reared in systems with temperature rates of approximately

16°C to 20°C, whereas *Capitella* sp. and *O. craigsmithi* show higher suitability for open-water IMTA systems at greater depths where temperature is stable at ~8°C. High abundance observed for *craigsmithi* on hard substrates in Norwegian waters indicates a potential competitive advantage in these environments (Hansen et al., 2011; Brennan, 2018). This is opposite to *Capitella* sp., which is mainly found in soft sediment. Aspects such as substrate preference are crucial when considering future technical development to the chosen polychaete in open-water IMTA. Both *Pereneris* sp. and *H. diversicolor*, belonging to the Neridae family, have a semelparous reproduction strategy, which has been pointed out as a challenge for maintaining stable stocking densities within IMTA system (Prevedelli and Cassai, 2001; Wang et al., 2020). In contrast, *O. craigsmithi* and *Capitella* sp. have iteroparity and provide the advantage of continuous recruitment. All polychaetes considered in the framework comprise valuable contents of fatty acids and essential amino acids when feed with aquaculture waste, making them attractive as a raw material in

TABLE 1 Framework for evaluating the suitability of deposit feeding polychaetes as candidate species in IMTA, based on a set of IMTA suitability indicators.

	IMTA suitability indicator	<i>Ophryotrocha</i> spp.	<i>Capitella</i> spp.	<i>Hediste diversicolor</i>	<i>Perineris</i> spp.
Environmental suitability	Cope with high organic loading	Proven to feed on fish wastes ^{4,9}	Proven to feed on fish wastes ^{4,7}	Proven to fed on fish waste ⁵	Proven to fed on fish waste ¹¹
	Cope with anoxic conditions	Observed underneath fish farms ^{1,2,3}	Observed underneath fish farms for temporal water species ⁷	Cope well with variation in salinity and oxygen concentrations ¹⁰	Observed under fish farms ¹¹
Biological suitability	High densities	Estimated densities of natural populations at fish farms of 100,000 individuals per square meter ³	Cultivated densities at fish farms of 134,000 individuals per square meter ⁷	Cultivated densities in RAS IMTA system up to 7,000 individuals per square meter ¹⁵	Densities at fish farms of 500–1,000 individuals per square meter ¹¹
	High growth rates*	SGR = 3.56% ± 0.3% day ⁻¹ (fresh salmon feces) ⁴	SGR = 2.56% ± 0.34% day ⁻¹ (fresh salmon feces) ⁴	SGR = 2.5% day ⁻¹ ⁵ fish feed SGR = 1.2% day ⁻¹ ⁵ smolt waste ⁵	SGR 0.64 ± 0.04% day ⁻¹ ¹⁴
	Iteroparous reproduction	Yes ¹³	Yes ⁸	No ¹⁷	No ¹⁶
Carbon and nitrogen mitigation potential	High turnover ratios	Based on carbon and nitrogen turnover measurements (26 mg C g ⁻¹ AFDW day ⁻¹ , 6 mg N g ⁻¹ AFDW day ⁻¹) ¹⁸ *Absorption efficiency unknown	Based on carbon and nitrogen turnover measurements (16 mg C g ⁻¹ AFDW day ⁻¹ , 3 mg N g ⁻¹ AFDW day ⁻¹) ¹⁸	Based on 70% reduction in organic material in IMTA cultivation system ¹⁵ and estimates provided by dynamic energy budget modeling ¹⁹	Based on high feeding and apparent digestibility rates. Remove > 50% of C and N from deposited material ¹¹
Market potential	High polychaete product potential	Total FA 104 g kg ⁻¹ AFDW ⁴ Rich in essential amino acids ⁴ Products not developed yet	Total FA 97 g kg ⁻¹ AFDW ⁴ Rich in essential amino acids ⁴ Products not developed yet	Total FA 57–74 g kg ⁻¹ DW ⁵ Total AA 285.3–310.8 g kg ⁻¹ DW ⁵ Products developed (bait, shrimp feed)	High on total fat content when reared on aquaculture waste, highest in large individuals and for systems with low densities ¹² High protein content, highest for small individuals ¹² Products developed (bait, shrimp feed)
Challenges		Mucus production	Infauna	All year-round access to juveniles ⁶ and control of reproduction ¹⁷	Infauna

¹ Eikje (2013), ² Hamoutene et al. (2013), ³ Paxton and Davey (2010), ⁴ Nederlof et al. (2019), ⁵ Wang et al. (2019), ⁶ Wang et al. (2020), ⁷ Kinoshita et al. (2008), ⁸ Ramskov and Forbes (2008), ⁹ Salvo et al. (2015), ¹⁰ Fidalgo e Costa et al. (1998), ¹¹ Fang et al. (2017), ¹² Palmer et al. (2014), ¹³ Present study, ¹⁴ Fang et al. (2016), ¹⁵ Marques et al. (2017), ¹⁶ Prevedelli and Cassai (2001), ¹⁷ Nesto et al. (2018), ¹⁸ Nederlof et al. (2020), ¹⁹ Galasso et al. (2020). *Growth rates should not be compared directly due to their dependency on initial animal size and environmental conditions.

aquatic feed. However, today, only *Perineris* sp. and *H. diversicolor* have a market potential due to their use as bait or as a component in shrimp feed (Olive, 1999; Palmer et al., 2014).

5 Conclusion

This study shows that *O. craigsmithi* displays multiple life history traits that are of benefit for farming and harvesting populations in benthic IMTA. They obtained high growth rates when fed with fish feed and have short a short generation time with multiple spawning events throughout their lifetime. This implies a high population growth and opens up for the possibility of multiple polychaete harvests during a single fish production cycle. Furthermore, these are gonochoric, and eggs are typically 120–150 µm in diameter. This species can initiate reproduction at half of their adult length, and fertilized eggs develop into free-swimming larvae after approximately 1 week, but further studies are needed to confirm this and to provide details for development past the free-swimming larvae stage. Life history traits are in line with what has been reported by studies on other members of the genus occupying shallower and warmer waters, but *O. craigsmithi* stands out as a large *Ophryotrocha* with broadcasting spawning. The described life history traits together with the presented framework, assessing different polychaete species suitability in a benthic IMTA, suggest that *O. craigsmithi* is an efficient, and well-suited extractive species for this purpose. Market potential remains to be realized, and future research should focus on further assessing the biomass and harvest potential in an open-water IMTA setting.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

SS: First authorship with main responsibility for planning for experiments, collection and processing of data; main author on writing the current manuscript. TS: second author; key role in fieldwork and planning of the experiment; has contributed with guidance and editing in the manuscript. AG: third author, assisting in analyses of the data, especially for applying the von-bertalanffy model; provided feedback on the current manuscript. HR: third author, assisting in laboratory work, especially during growth trial and larvae development; feedback in the current manuscript. E-JL: Fourth author, provided feedback during the editing manuscript. HS:

Fifth author, provided feedback during the editing manuscript. HJ: Senior authorship, she has made a great contribution to editing this manuscript and was involved during the planning and at some data sampling points during data collection in the laboratory. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors SS and HS are employed in Lerøy seafood group.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1116765/full#supplementary-material>

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