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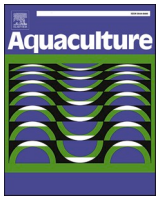

# Diet rather than temperature determines the biochemical composition of the ragworm *Hediste diversicolor* (OF Müller, 1776) (Annelida: Nereidae)

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ABSTRACT

The polychaete *Hediste diversicolor* is known to recycle side streams from aquaculture and biogas production. We conducted a feeding experiment to evaluate whether rearing temperature or mixtures of these two side streams enhances biomass production and fatty acid composition. We reared *H. diversicolor* along a 5-temperature gradient ranging from 5.8 °C to 17.1 °C and a 4-step gradient form 100% aquaculture sludge to 100% solid biogas digestate. Formulated fish feed served as a control diet. Polychaetes increased growth rate with increasing temperature, ranging from 0.01 at 5.8 °C to 0.14 at 17.1 °C, while survival was inversely affected by temperature with 100% survival at 5.8 °C and 70% survival at 17.1 °C. Diet had a less pronounced effect on polychaete survival, and no significant effect on growth rates. Contrasting to growth, the fatty acid composition of the polychaetes was not affected by temperature but was highly influenced by diet, as polychaetes did not cluster by rearing temperature but by the diets they received. In conclusion, *H. diversicolor* can be utilized as a recycler of aquaculture and biogas side streams, and production temperature can be optimized for growth without compromising fatty acid composition and quality of the polychaetes.

## 1. Introduction

There is an urgent need to change our economy from linear production systems toward circular, recycling based solutions. Many wastes or rest materials from linear systems are in fact valuable side streams containing compounds too precious to be incinerated or end as landfills. Sludge from land-based aquaculture is such a side stream, which is comprised of faeces and uneaten pellets. For Atlantic salmon (*Salmo salar*), depending on the precision of the feeding and the fish's appetite, 3–20% of the pellets end up uneaten (Aas and Åsgård, 2017). Further, fish digestion is far from perfect, and 20% of the energy and 18% N and 50% P taken up by the fish is released as solids (Wang et al., 2012). In total, up to 70% of the phosphorus, 60% of the energy and about 50% of PUFAs provided to the fish end up uneaten in the sludge (Ytrestøyl et al., 2015). Nowadays, if recycled at all, solid side streams from aquaculture are used as fertilizers for agriculture (Brod et al., 2017; Wang et al., 2012). A second readily available side stream is the solid phase remaining after biogas production commonly known as "solid biogas digestate" (SBD). Theoretically, most of the energy and nutrients contained in the feed stock supplied to a biogas digester is used for bacterial biomass production and methanogenesis. However, the efficiency of digestors is also not perfect, with about 50–80% of the organic matter being converted to biogas and therefore implying that 20–50% of the feed stock channelled into biogas production remains in the digestate. The remaining nutrients in the digestate are usually used as agriculture fertilizer, where the focus is rather on dissolved inorganic nutrients than on organic compounds present in the solid phase, hence using the solid phase of biogas digestate (SBD) for other applications would not even be in competition to the traditional users. Aquaculture sludge (AS in the following) and solid biogas digestate (SBD in the following) have in common that they are still nutrient and energy rich and must be collected and handled anyway at the production sites, hence the

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question arises if these side streams can be utilized in a more sustainable way to recycle nutrients and energy and create value.

Polychaete worms, particularly *Hediste diversicolor*, are promising candidates to recycle side streams such as AS and SBD. They are omnivorous and have various feeding strategies, from actively searching for detritus to casting a mucous net to capture very small particles such as phytoplankton (Riisgård, 1994). Fish faeces naturally form a part of the detritus polychaetes are living off in nature, and indeed, these polychaetes have been shown to process AS into biomass (Bischoff et al., 2009; Wang et al., 2019b). Polychaetes also harbour bacteriolytic enzymes, which may allow them to further digest SBD, which mainly consists of bacteria. Previous studies have suggested that nereid polychaetes thrive on SBD was published (Wang et al., 2019a), and further investigations are a promising research topic. Both SBD and aquaculture sludge have been shown to be suitable feeds for polychaetes, but the growth rates were lower than those fed high quality diets such as formulated fish feed (Wang et al., 2019a). This finding clearly shows that certain compounds were either missing, or not present in the appropriate concentrations or proportions to suit the polychaetes demand. Demand changes from energy rich diet at higher temperatures to account for an increased basal metabolism, toward a more nutrient-rich diet (Malzahn et al., 2016). Additionally, polyunsaturated fatty acids (PUFA) rich diets are favourable at low temperatures to account for the decrease in routine metabolism and an increased demand for (PUFA) to counteract the cold-induced decrease in membrane fluidity (Hall et al., 2002).

Both side streams differ in biochemical composition depending on many factors. Biogas digestate quality depends on feed stock for the digester, and efficiency of the digester (Al Seadi et al., 2013), while aquaculture sludge differs depending on farm type, fish species, life stage of fish, and sludge management (Chen et al., 1997; Cripps and Bergheim, 2000). Wang et al. (2019a) reported that SBD from the same biogas plant the SBD originated in the current study was lacking virtually all n-3 fatty acids, only containing traces of DHA, and contained high levels of saturated fatty acids, manly 16:0 and 18:0. In contrast, aquaculture sludge in the Wang publication contained higher levels of 18:1 n-9 and n-3 fatty acids and less saturated fatty acids. Hence, a combination of both side streams might provide a better food quality at a given temperature and might support growth rates beyond single diets.

Polychaete worms are further interesting organisms, as there are already established markets for different worm products. Among them is the bait market supplying anglers with bait, a huge demand by the shrimp industry where polychaetes are a central ingredient in maturation diets (Chimsung, 2014; Leelatanawit et al., 2014; Luis and Ponte, 1993; Yang et al., 2022), and polychaetes also serves as an ingredient in specialized fish feeds.

A central reason for polychaetes being discussed as a future feed ingredient is their biochemical profile (Barroso et al., 2013; Nederlof et al., 2019; Stabili et al., 2019), suiting the nutritional demand of marine fishes (Amran and Mohamad, 2022; Olive, 1999). Contrary to e.g. insects, which are also discussed as a currently used feed ingredient, polychaetes are rich in high nutritional value long-chain ($\geq C_{20}$) PUFA including high levels of the omega-3 fatty acids eicosapentanoic acid (EPA) and docosahexaenoic acid DHA (Bischoff et al., 2009; Wang et al., 2019a; Wang et al., 2019b). These compounds are essential for fish, hence must be supplied with the feed to prevent deficiency symptoms (Sargent et al., 1995). EPA and DHA, among other PUFA, play major roles in many biological processes, such as being precursors of the biologically active eicosanoids, and ensuring normal brain function and vision. Importantly, PUFA in general can contribute to keeping membrane fluidity at low temperatures through an adaptive response known as "homeoviscous adaptation" (HVA) (Sinensky, 1974). HVA enables organisms to change the degree of unsaturation of fatty acids in cell membranes to maintain a certain viscosity of their membranes. Regulation of fatty acid concentrations in relation to temperature has been reported for a whole suit of poikilotherms, such as insects (Joanisse and Storey, 1996; Lehmann et al., 2020), molluscs (Hall et al., 2002), and fish (Laurel et al., 2012; Malekar et al., 2018). Within the phylum Annelida, information on HVA is scarce. The terrestrial worm *Dendrobaena octaedra* has been shown to accumulates PUFA under cold adaptation (Holmstrup et al., 2007). Similar results have been reported from the marine reef building sabellid polychaete *Sabellaria alveolate* when incubated at a range of temperatures (Muir et al., 2016).

The aims of this study were to investigate if (A) *H. diversicolor* thrives on the two side streams aquaculture sludge and solid biogas digestate, (B) if the nutritional demand of the polychaetes changes with temperature and hence the effect of the different diets on growth also changes with temperature, and (C) if *H. diversicolor* displays HVA as a response to low temperatures by an increase of PUFA concentrations, which can be utilized to increase PUFA recycling and endogenous production.

## 2. Materials & methods

To investigate the effects of temperature and diet composition on growth and biochemical composition of the polychaete *H. diversicolor*, worms were fed along a 5-step temperature gradient from 5.8 to 17.1 °C and a 4-step feed gradient and from 100% SBD to 100%AS.

### 2.1. Experimental design

Wild polychaetes (*H. diversicolor*) were collected on the 4.12.2020 at low tide at the mud flat of Leangen Bay, Trondheim, Norway (63°26′24.5″N, 10°28′27.7″E). Polychaetes were transported to the laboratories of SINTEF Ocean and stored in flow-through tanks at 10 °C until the start of the experiment. During this period the polychaetes were fed on commercial fish feed.

### 2.2. Feed preparation

To prepare the experimental diets, side streams were processed as follows. SBD was received from Biokraft AS, Trondheim, Norway. As SBD can contain high concentrations of ammonia (Al Seadi et al., 2013), the solid residual was resuspended in distilled water, centrifuged and the supernatant was discarded. This procedure was repeated three times and the sediment was frozen for later use. AS was received from a salmon smolt producer (Lumarine AS, Tjeldbergodden). Upon arrival, AS was allowed to settle, the sediment was frozen for later use and the supernatant was discarded. To determine the proximate composition the diets, AS and SBD was freeze dried, and analyzed for dry matter, ash, amino acids, and the content of carbon and nitrogen. Ash was determined by weighing the freeze-dried sample before and after combusting the samples at 450 °C in a muffle furnace for 5 h. Amino acids were analyzed using HPLC (Agilent Technologies Infinity 1260) coupled with an online post-column derivatization module (Pinnacle PCX, Pickering laboratories, Mountain View, CA, USA). Freeze-dried samples were hydrolysed in 6 M HCl containing 0.4% mercaptoethanol at 110 °C for 24 h. After hydrolysis, samples were filtered (Whatman GF/C, 47 mm) and pH was adjusted to 2.2 before HPLC analysis. Protein content was calculated from the sum of water-free total AAs (TAAs) divided by a factor of 0.9 to account for amino acids that are not fully analyzed by HPLC—such as cysteine and tryptophan (Øie and Olsen, 1997), since these have been estimated to account for approximately 10% of the TAAs (Watanabe et al., 1983). Carbohydrates were calculated by subtracting ash, protein and lipid content (avg. values) from the total dry weight of the samples. The results (Table 1) allowed calculating the necessary amounts of feed for the experiment. The amount of feed needed was calculated to match 30% of the polychaetes estimated nitrogen content per day. Based on previous experiments (Wang et al., 2019a), polychaetes were assumed to contain 8.6% nitrogen of dry matter, and water content of polychaetes was assumed to be 80%. Polychaetes were weighed and the amount of nitrogen was calculated for the 4 diets: 100% AS, 66%AS/33%SBD, 33%AS/66%SDB, and 100%

**Table 1**
Proximate composition of the different side streams, shown as either % dry matter (DM), mg $g^{-1}$ of DM or % wet weight (WW). All values are averages of three analysis ± standard deviation.

| Component | Aquaculture sludge | Solid Biogas Digestate |
|---|---|---|
| C (mg $g^{-1}$ DM) | 117.46 ± 11.81 | 311.41 ± 11.72 |
| N (mg $g^{-1}$ DM) | 15.08 ± 3.04 | 47.90 ± 2.06 |
| Moisture (% WW) | 58.90 ± 0.39 | 80.47 ± 0.43 |
| Ash (% DM) | 29.76 ± 0.27 | 9.05 ± 0.17 |
| Protein (% DM) | 8.41 ± 0.19 | 20.91 ± 0.76 |
| Lipids (% DM) | 4.17 ± 0.01 | 6.70 ± 0.05 |
| Carbohydrates (% DM)* | 57.66 | 63.34 |

* Carbohydrate content was calculated from mean ash, protein, lipid, and dry weight.

SBD. The respective amounts of AS and SBD were carefully weighed into 1.5 mL tubes for each experimental container and each feeding event and subsequently frozen until fed to the polychaetes.

### 2.3. Experimental procedures

Prior to the experiment polychaetes were allowed to evacuate their guts in cups containing sea water and no sediment for 4 h, which has been shown to be sufficient to achieve empty guts in *H. diversicolor* (Wang et al., 2019b). Polychaetes were then individually weighed in a tared beaker containing tempered sea water, and 7 individuals were stocked into each experimental container. The bulk weight of each replicate was used to calculated daily feed rations for each container. Experimental containers consisted of 800 mL glass beakers (diameter 95 mm) containing around 200 mL of sand, resulting in an approx. 8 cm thick sediment layer.

The experiment was conducted in a temperature gradient device modified after Thomas et al. (1963). The device is an aluminium plate which cooling on one, and heating on the other side, creating a very stable temperature gradient over the plate. It contains drill holes snugly fitting 800 mL standard laboratory glass beakers. The device has 10 different temperature zones and 6 spaces within each temperature zone. The temperature on both sides of the temperature gradient table was set to 10 °C at the beginning of the experiment. After 24 h the low temperature was adjusted to 4 °C, and the high temperature was set to 20 °C, resulting in experimental temperatures of 5.8 °C, 9.5 °C, 12.5 °C, 14.7 °C, and 17.1 °C. Throughout the experiment, water was exchanged daily, and polychaetes were fed every other day.

The experiment was conducted in a full factorial design. At each temperature, one glass beaker containing 7 polychaetes was assigned a feeding treatment, leading to a full factorial design with five temperatures and five different feed types at each temperature (five temperatures x five feed types = 25 experimental units).

After 15 d (7 feedings), polychaetes were dug out of the sediment and allowed to evacuate their guts for 4 h as described above. All polychaetes were weighed individually for wet weight as described above to estimate growth performance. Moreover, three polychaetes from each experimental container were randomly sampled for total lipids and fatty acid analyses. These were frozen in a nitrogen atmosphere at −80 °C and subsequently freeze dried.

### 2.4. Growth performance

Specific growth rates (SGR) were calculated using the average wet weight of the seven polychaetes of each replicate at the beginning of the experiment, and at the average wet weight of the remaining polychaetes at the end of the experiment. SGR was calculated as follows: SGR = (ln Wet weight $t_{end}$ – ln wet weight $t_0$)/ t, where $t_o$ and $t_{end}$ are the weight of the polychaetes at the beginning and at the end of the experiment.

### 2.5. Total lipids and fatty acid analysis

Total lipids and fatty acids were analyzed from three individual polychaetes per experimental container as well as from three samples of each experimental diet. Briefly, total lipids were extracted from the homogenized samples using the Folch's method (Folch et al., 1957). Subsequently, total lipids were used to prepare fatty acid methyl esters (FAME), which were analyzed using a Thermo Trace GC Ultra Gas Chromatograph (Thermo Electron Corporation, Waltham, MA, USA), equipped with a fused silica 30 m × 0.25 mm open tubular column (Tracer, TR-WAX, film thickness: 0.25 mm, Teknokroma, Sant-Cugat del Vallés, Spain), fitted with an on-column injection system, using helium as a carrier gas, and a flame ionization detector (FID). The analytical temperature was programmed from 50 °C to 220 °C. FAME peaks were integrated and analyzed with Azur Datlys (St Martin d'Heres, France) software. FAME were identified by comparison of retention times of each peak with those of well-characterized standards.

### 2.6. Data analysis

We performed a two-way ANOVA on growth using the built-in statistical package of SIGMA Plot 12.5. Principal component analysis (PCA) on fatty acid composition of polychaetes was performed using PRIMER 5.2. Fatty acid levels were expressed as % of total fatty acids and were arcsine transformed before entering the PCA.

## 3. Results

Growth of polychaetes was significantly influenced by temperature (two-way ANOVA, $p < 0.001$), but not by diet (two-way ANOVA, $p > 0.05$). Growth was positively related to temperature until 14.7 °C and declined with a further increase of the temperature till 17.1 °C (Fig. 1) (Table 2). The proportions of AS and SBD in the diet showed no significant effects on growth rate within temperatures. Temperature and feed type had a significant influence on polychaete survival (temperature p < 0.001, feed type $p < 0.05$, two-way ANOVA) (Table 2). Survival of polychaetes showed a clear temperature relationship with an increase in mortality with increasing temperature (Fig. 2). Compared to

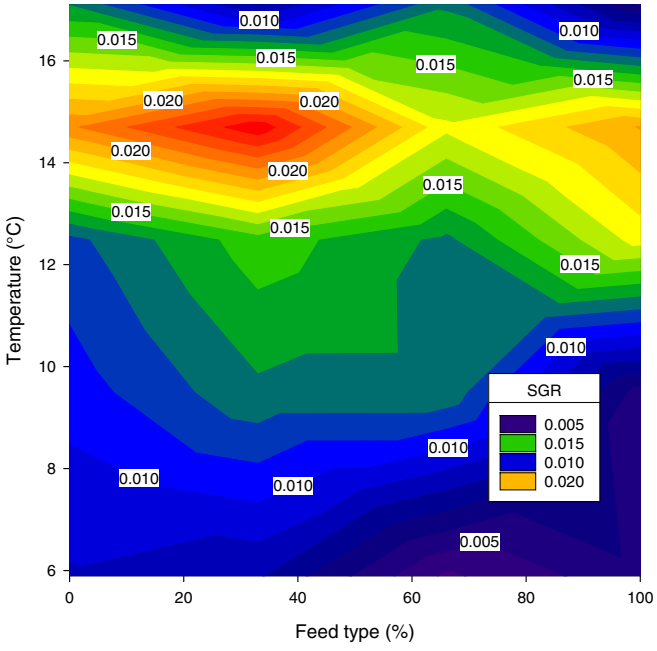


**Fig. 1.** Relationship between specific growth rate (SGR, %), feed type (% of aquaculture sludge in the diet), and temperature (°C) for *H. diversicolor* during the trials.

**Table 2**
Average survival and specific growth rates of *H. diversicolor* at the five experimental temperatures. Numbers are averages ± standard deviation. $n = 5$.

| Temperature | 5.8 °C | 9.5 °C | 12.5 °C | 14.7 °C | 17.1 °C |
|---|---|---|---|---|---|
| Survival (%) | 100 ± 0 | 97 ± 6.3 | 91 ± 7.8 | 80 ± 12.7 | 71.4 ± 10. |
| Specific growth rate | 0.007 ± 0.002 | 0.01 ± 0.003 | 0.03 ± 0.003 | 0.02 ± 0.003 | 0.14 ± 0.01 |

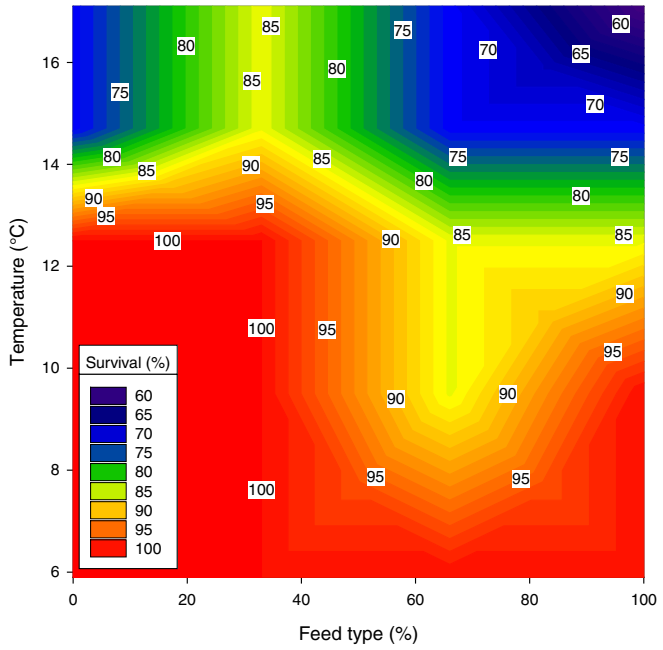


**Fig. 2.** Relationship between feed type (% of aquaculture sludge in the diet), temperature (°C) and survival for *H. diversicolor* during the trials.

temperature, feed type did not have such a pronounced, yet a significant effect on survival. Lower proportions of aquaculture sludge promoted survival.

The two side streams which served as the diets in the current experiment differed tremendously. SBD was richer in carbon, nitrogen, protein, and lipids, and contained less ash (Table 1). The fatty acid composition of the two side streams also differed in individual fatty acids (Fig. 3), but not so much when fatty acids are combined into categories such as saturated, mono-unsaturated and poly-unsaturated fatty acids (41, 42 and 16% of total fatty acids, respectively for both, SDB and AS, Table 3). The main differences between fish feed and the side stream diets were the high concentrations of EPA and DHA in fish feed (Table 3). EPA accounted for 15% of total fatty acids in fish feed, and about 1% in both, aquaculture sludge and solid biogas digestate. DHA accounted for 8.5% of total fatty acids in fish feed, while it accounted for 2% in aquaculture sludge and 3.5% in solid biogas digestate. Further, aquaculture sludge was richer in 16:0 (28% vs. 18% in solid biogas digestate and 20% in fish feed), and it was also richer in 18:1n-9 (25% vs. 21% in solid biogas digestate and 13% in fish feed). In sum, fish feed contained a lower percentage of saturated fatty acids compared to aquaculture sludge and digestate (32% vs. 41% in both, aquaculture sludge and biogas digestate), and in turn a higher percentage of unsaturated fatty acids.

The fatty acid composition of polychaetes fed side stream diets showed high concentrations of EPA (14–19%) comparable to the polychaetes fed fish feed (13%). Further, the percentage of PUFA was high in both (38–40%) also comparable to those fed fish feed (46%). However, DHA was higher in polychaetes fed fish feed (5%) than in polychaetes fed aquaculture sludge and SBD diets (1–2%) (Tables 4 & 5).

The pronounced differences in fatty acid composition found in the different feeds were not as pronounced in the polychaetes reared on these diets (Fig. 4, Tables 4 & 5). Arranging the plot of the principal component analysis on all fatty acids by the diets the polychaetes received, the only obvious grouping is that polychaetes receiving fish feed clustered. Polychaetes receiving solid biogas digestate, aquaculture sludge or the two mixes of the latter did not differ in their fatty acid composition. ANOVA revealed that polychaetes fed fish feed had significantly higher levels of DHA, and PUFA, while they contained lower percentages of saturated fatty acids ($p < 0.05$). Annotating the plot by rearing temperature revealed no pattern in the fatty acid composition of the polychaetes related to temperature. ANOVA revealed that none of the fatty acid measures which differed in the feeds, differed in the polychaetes.

## 4. Discussion

Aquaculture sludge widely differs in quality and composition due to feeding strategies, fish life stage, sludge collection technologies and sludge age (Aas and Åsgård, 2017; Cripps and Bergheim, 2000). The sludge used in the current experiment was of surprisingly low quality. It contained around 8% protein and 5% lipids, and a high proportion of ash (30%), suggesting a high degree of remineralization had already occurred during storage before being used for this experiment. Depending on the conditions, the composition and the availability and type of electron acceptors of the initial sludge, sludge decay constants can range from 0.024 to 0.006 $d^{-1}$ (van Rijn, 2013), especially when oxygen serves as the electron acceptor. When the supernatant of sludge thickening tanks was kept aerobic, 87% of the organic matter was remineralized within 6 days (Brazil and Summerfelt, 2006). Even under anaerobic conditions 30–40% of the sludge can be remineralized within <2 weeks (Klas et al., 2006). Hence, using aquaculture sludge as a feed input for secondary bioproduction calls for a good sludge management with short retention times in filters or settlement tanks, and a rapid processing to maintain the original quality of the sludge. The quality and composition of SBD largely depends on the feedstock used as input for the biogas production (Al Seadi et al., 2013), and many countries have positive lists in place which regulate the feedstocks allowed to be used in biogas production. Further, processing protocols and technologies are developed, as processed SBD is an established product for e.g. soil conditioning and fertilization (Al Seadi et al., 2013). Usually around 50% of the dry matter is converted to biogas during digestion, but such conversion can go up to 90% when energy crops such as grass or corn are used as feedstock (Murphy et al., 2011), meaning that the quality of the digestate as e.g. feed for polychaetes varies considerably depending on the management of the biogas plant. Hence, side streams might serve as feed input for worm production directly or could be mixed to achieve a sufficient quality as a diet for polychaete production.

Polychaetes fed such sludge and SBD in the present study grew according to expectations, and were comparable to previous reports by Wang et al. (2019a) and Wang et al. (2019b), who reported that polychaetes grew on different side streams, but not as fast as polychaetes fed formulated fish feeds. Pajand et al. (2017) reported remarkably higher growth rates for the same polychaete species reared on sturgeon sludge but cultivated their polychaetes at 23 °C and started with very small individuals, where growth rates are generally higher. Further, the population of *H. diversicolor* used in the Pajand study stems from the Caspian Sea, and is genetically distinct from the Norwegian population used in the current study (Teixeira et al., 2022; Virgilio et al., 2009), and hence might differ in many traits, such as optimum temperatures for growth. Similar high growth rates have been reported by Gómez et al. (2019) who cultivated *Abarenicola pusilla* on sludge from *Seriola lalandi* recirculating aquaculture system (RAS) cultivation. The authors reported a high bioremediation potential, and growth rates of up to 3% per day. In sum, polychaetes are capable of recycling side streams into biomass. Growth in poikilotherms usually increases with temperature within

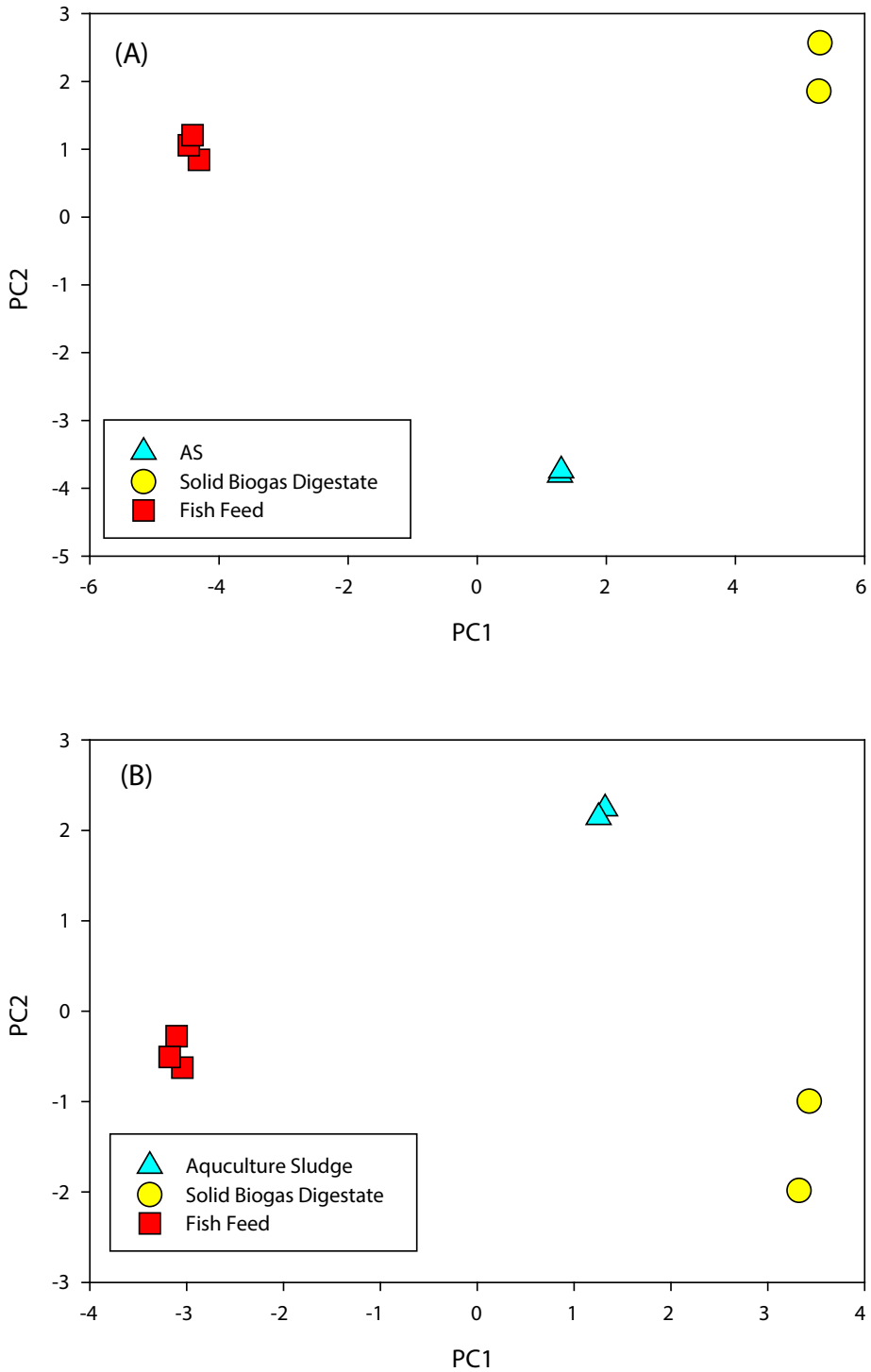


**Fig. 3.** Principal Component Analysis (PCA) on total FA (A) and PUFA (B) composition of different diets used during the feeding experiment. FF = Fish feed, SBD = Solid Biogas Digestate, and AS = Aquaculture Sludge. (A) PC1 = 72.6%, PC2 = 25.8% (B) PC1 = 76%, PC2 = 21%.

biological limits, and decreases beyond a certain temperature. The mechanism behind such decrease in growth is most likely associated with decreased oxygen transport capacity at high temperatures (Pörtner and Knust, 2007). Increases in growth in *H. diversicolor* between 5 and 20 °C was also reported by Olive et al. (1997) who investigated interactions between temperature and light period on growth of *H. diversicolor*. In our study, growth rate increased up to 15 °C, beyond which growth rates decreased again and as pointed out above, oxygen deficiency being the most likely explanation for the decrease.

The fatty acid composition of the polychaete biomass produced seemed to be largely independent of the rearing temperature but highly influenced by the fatty acid composition of the diet. The fatty acid composition of polychaetes fed fish feed differed from those of polychaetes fed aquaculture sludge or solid biogas digestate, the main fatty acids driving these differences being higher concentrations of DHA and 18:2n-6, and lower concentrations of 22:4 n-6 and 22:5 n-3. Theoretically, we would have expected a response of the fatty acid composition to temperature in line with homeoviscous adaptation theory (HVA), which predicts that the fatty acid composition of cold adapted organisms has a higher unsaturation index. This would be also supported by the

**Table 3**
Fatty acids of diets used to rear *H. diversicolor*. Data are % of total fatty acids (average +/− standard deviation in brackets, $n = 3$). Values mentioned in the text are highlighted in bold.

| | Diets | | |
|---|---|---|---|
| Fatty acid | Fish feed | SBD | AS |
| 14:0 | 7.49 (0.04) | 5.07 (0.06) | 4.77 (0.04) |
| 15:0 | 0.36 (0.02) | 1.29 (0.07) | 0.48 (0) |
| 16:0 | **20.95 (0.05)** | **18.62 (0.26)** | **28.47 (0.13)** |
| 16:1 | 7.79 (0.09) | 4.18 (0.06) | 2.68 (0) |
| 17:0 | 0 (0) | 2.76 (0.13) | 0.34 (0.01) |
| 18:0 | 3.35 (0.02) | 9.52 (0.11) | 5.67 (0.03) |
| 18:1n9 | **13.5 (0.05)** | **21.09 (0.07)** | **24.67 (0.04)** |
| 18:1n7 | 3.35 (0.01) | 4.92 (0.03) | 4.61 (0.11) |
| 18:2n6 | 7.77 (0.05) | 6.58 (0.25) | 7.66 (0.02) |
| 18:3n3 | 1.34 (0.01) | 2.43 (0.06) | 1.86 (0.02) |
| 18:4n3 | 0 (0) | 0.58 (0.04) | 0.51 (0) |
| 20 0 | 0.26 (0.01) | 1.9 (0.07) | 0.62 (0.01) |
| 20:1 | 4.87 (0.3) | 3.52 (0.01) | 4.94 (0.02) |
| 20:2n6 | 0.16 (0.01) | 0.34 (0.07) | 0.41 (0.01) |
| 20:3n6 | 0.08 (0.01) | 0.13 (0.01) | 0.27 (0.01) |
| 20:4n6 | 0.9 (0.05) | 0.22 (0.02) | 0.66 (0) |
| 20:3n3 | 0.08 (0.01) | 0.2 (0.08) | 0.23 (0) |
| 20:4n3 | 0.58 (0.01) | 0.23 (0.03) | 0.54 (0) |
| 20:5n3 | 14.17 (0.12) | 1 (0.09) | 1.17 (0.02) |
| 22:0 | 0.11 (0.02) | 2.16 (0.06) | 0.74 (0.04) |
| 22:1n11 | 0 (0) | 2.13 (0.17) | 4.15 (0.03) |
| 22:1n9 | 2.19 (0.29) | 0.73 (0.01) | 0.55 (0.01) |
| 22:4n6 | 0 (0) | 0.19 (0.06) | 0.06 (0) |
| 22:5n3 | 1.3 (0.04) | 0.22 (0.04) | 0.88 (0.01) |
| 22:6n3 | 8.72 (0.04) | 3.51 (0.06) | 1.99 (0.03) |
| saturated FA | **32.53 (0.15)** | **41.49 (0.48)** | **41.08 (0.15)** |
| monounsaturated FA | 32.37 (0.46) | 42.88 (0.09) | 42.69 (0.15) |
| polyunsaturated FA | **35.1 (0.31)** | **15.63 (0.39)** | **16.24 (0)** |

**Table 4**
Fatty acids of *H. diversicolor* grouped by temperature. Data are % of total fatty acids (average +/− standard deviation in brackets, n = 3).

| | | | Polychaetes by temperature | | |
|---|---|---|---|---|---|
| Fatty acid | 5.9C | 9.5 °C | 12.5 °C | 14.7 °C | 17.1 °C |
| 14:0 | 1.88 (0.51) | 1.89 (0.32) | 2.07 (0.95) | 1.67 (0.74) | 1.85 (0.73) |
| 15:0 | 0.68 (0.12) | 0.69 (0.13) | 0.68 (0.18) | 0.63 (0.14) | 0.63 (0.23) |
| 16:0 | 18.32 (2.38) | 18.78 (1.77) | 19.18 (2.95) | 18.59 (2.14) | 19.38 (2.24) |
| 16:1 | 5.05 (1.37) | 4.76 (1.07) | 3.85 (1.57) | 4.13 (1.18) | 3.96 (1.42) |
| 17:0 | 0.97 (0.23) | 1.05 (0.32) | 1.02 (0.31) | 1.03 (0.38) | 1.06 (0.46) |
| 18:0 | 5.32 (0.85) | 5.08 (0.76) | 5 (0.78) | 5.39 (1.18) | 5.49 (1.24) |
| 18:1n9 | 12.98 (4.86) | 11.3 (3.78) | 12.98 (2.86) | 12.93 (3.75) | 12.07 (2.8) |
| 18:1n7 | 4.18 (1.04) | 5.08 (1.07) | 4.29 (0.78) | 4.37 (0.98) | 4.45 (0.8) |
| 18:2n6 | 9.13 (5.44) | 6.33 (3.75) | 9.77 (6.2) | 9.32 (6.03) | 6.88 (3.07) |
| 18:3n3 | 2.09 (0.62) | 2.76 (2.09) | 3.13 (2.38) | 2.01 (0.95) | 2.08 (0.91) |
| 18:4n3 | 0.31 (0.23) | 0.51 (0.6) | 0.63 (0.84) | 0.38 (0.34) | 0.44 (0.35) |
| 20 0 | 0.12 (0.07) | 0.09 (0.07) | 0.12 (0.09) | 0.16 (0.14) | 0.14 (0.07) |
| 20:1 | 8.04 (1.27) | 8.51 (1.3) | 7.21 (1.83) | 8.26 (1.65) | 8.55 (1.34) |
| 20:2n6 | 3.84 (1.1) | 3.24 (0.62) | 3.23 (0.79) | 3.23 (1.13) | 3.17 (0.91) |
| 20:3n6 | 0.52 (0.27) | 0.37 (0.19) | 0.44 (0.26) | 0.74 (0.74) | 0.4 (0.19) |
| 20:4n6 | 2.54 (1.01) | 1.91 (0.52) | 2.08 (0.69) | 2.1 (0.93) | 2.53 (1.08) |
| 20:3n3 | 0.54 (0.16) | 0.66 (0.32) | 0.57 (0.27) | 0.49 (0.22) | 0.56 (0.22) |
| 20:4n3 | 0.39 (0.19) | 0.57 (0.57) | 0.58 (0.59) | 0.42 (0.22) | 0.4 (0.22) |
| 20:5n3 | 15.43 (3.93) | 17.12 (3.84) | 14.7 (4.36) | 14.83 (4.45) | 16.25 (3.47) |
| 22:0 | 0.11 (0.07) | 0.07 (0.06) | 0.08 (0.09) | 0.08 (0.1) | 0.1 (0.09) |
| 22:1n11 | 1.24 (0.87) | 1.91 (0.91) | 2.47 (1.14) | 2.29 (2.07) | 2.7 (2.24) |
| 22:1n9 | 0.08 (0.06) | 0.09 (0.07) | 0.08 (0.09) | 0.08 (0.09) | 0.12 (0.15) |
| 22:4n6 | 1.56 (0.99) | 1.66 (0.68) | 1.04 (0.64) | 1.32 (0.93) | 1.62 (0.76) |
| 22:5n3 | 2.38 (1.2) | 3.13 (1.07) | 2.17 (1.09) | 2.45 (1.02) | 2.65 (0.93) |
| 22:6n3 | 2.1 (2.18) | 2.36 (1.44) | 2.49 (1.9) | 2.98 (2.88) | 2.39 (2.21) |
| saturated FA | 27.27 (3.29) | 27.55 (2.53) | 28.04 (4.48) | 27.39 (3.8) | 28.51 (3.55) |
| monounsaturated FA | 31.78 (3.44) | 31.75 (2.79) | 31 (3.96) | 32.19 (4.46) | 31.98 (4.17) |
| polyunsaturated FA | 40.95 (4.16) | 40.7 (3.6) | 40.96 (6.63) | 40.42 (5.63) | 39.51 (3.97) |

fact that polychaetes including *H. diversicolor* (Kabeya et al., 2020) have active fatty acid desaturation systems enabling them to counteract the cold temperatures to maintain cell membrane fluidity (Kabeya et al., 2020; Monroig and Kabeya, 2018). Muir et al. (2016) found evidence for HVA in *Sabellaria alveolata,* a reef building sabellid polychaete. Warm adapted populations had a lower unsaturation index compared to cold adapted populations, and cold adapted ones quickly decreased their unsaturation index when being exposed to warmer temperatures (Muir et al., 2016). However, EPA, for instance, showed no response in northern, cold adapted *S. alveolata* populations, these polychaetes kept their EPA concentrations at a constant level, irrespective of temperature. The absence of HVA in our study matches the findings of Sandmann (2019). Sandmann also reported no signs of temperature dependent changes in fatty acid composition. One could speculate that the experimental duration of 15 d (present study) or 4 weeks (Sandmann experiment) was not long enough to pick up HVA, but the paper of Muir et al. (2016) includes a heat map of changes in fatty acid composition over a temperature increase, where differences are already visible at a 5 °C temperature change after 4 days.

HVA is certainly a common adaptation to temperature changes, but HVA may be not as common and straight-forward as the literature suggests. Rais et al. (2010) found no trace of homeoviscous adaptation in the intertidal gastropods *Tegula funebralis* and *Littorina keenae.* The authors speculate that not membrane fluidity itself is regulated, but the internal milieu of the organism is adapted to changes in the exterior temperature, e.g., pH is adjusted, resulting in changes in membrane fluidity. The lack of adaptation might also be explained by poikilotherms need to be more flexible about membrane fluidity, especially those exposed to fluctuating temperatures at short timescales, such as tidal cyclicity or day-night rhythms. Hazel and Williams (1990) reported that different tissues within an animal react differentially, and some tissues show no adaptation. This is related to the environment these tissues are located in- e.g., tissues in hostile acidic environments for brush border membranes. Rais et al. (2010) also speculated about differential HVA response of different tissues as they used mantle and gill tissue in their study on snails, and reasoned that one might show HVA but not the other, thus one camouflaging the other tissues response.

Further, Hazel and Williams (1990) suggested that HVA might not really be a fine-tuning mechanisms within a given temperature range, but more a mechanism to surely stay in the fluid phase over a wider range of temperatures, which would then be *homeophasic* adaptation. Hence, assuming adjustments of fluidity by an increase of PUFA in polychaetes reared at low temperatures as a general rule might simply be incorrect, as the fluidity of membranes may be not regulated in such tight temperature ranges in all species but might be adjusted to the temperature range the polychaetes usually experience within biological limits.

**Table 5**
Fatty acids of *H. diversicolor* grouped by diets. Data are % of total fatty acids (average +/− standard deviation in brackets, n = 3). Values mentioned in the text are highlighted in bold.

| | | | Polychaetes by diet | | |
|---|---|---|---|---|---|
| Fatty acid | 100/0 | 66/33 | 33/66 | 0/100 | Fish feed |
| 14:0 | 1.75 (0.67) | 1.95 (1.1) | 2.02 (0.47) | 1.95 (0.5) | 1.67 (0.43) |
| 15:0 | 0.69 (0.13) | 0.6 (0.19) | 0.71 (0.1) | 0.77 (0.12) | 0.54 (0.16) |
| 16:0 | 19.17 (2.33) | 18.97 (2.56) | 20.33 (1.59) | 19.01 (1.51) | 16.78 (2) |
| 16:1 | 4.32 (1.16) | 4.08 (1.71) | 4.85 (1.33) | 4.8 (1.4) | 3.7 (1.04) |
| 17:0 | 1.04 (0.29) | 0.97 (0.25) | 1.12 (0.4) | 1.22 (0.24) | 0.79 (0.36) |
| 18:0 | 5.18 (0.89) | 5.31 (0.87) | 5.65 (0.98) | 5.69 (0.85) | 4.46 (0.85) |
| 18:1n9 | 12.28 (3.41) | 13.51 (5.62) | 12.14 (2.95) | 11.05 (2.98) | 13.27 (2.34) |
| 18:1n7 | 4.78 (0.85) | 4.56 (1.32) | 4.52 (0.71) | 4.61 (0.99) | 3.88 (0.72) |
| 18:2n6 | 5.99 (1.8) | 6.75 (3.01) | 7.34 (1.99) | 5.59 (2.29) | 15.75 (6.35) |
| 18:3n3 | 2.93 (2.69) | 2.64 (1.89) | 2.29 (0.83) | 1.8 (0.64) | 2.42 (0.75) |
| 18:4n3 | 0.7 (0.88) | 0.49 (0.62) | 0.38 (0.22) | 0.32 (0.24) | 0.39 (0.24) |
| 20 0 | 0.15 (0.14) | 0.13 (0.08) | 0.14 (0.08) | 0.12 (0.08) | 0.1 (0.06) |
| 20:1 | 8.66 (1.4) | 8.21 (1.11) | 8.68 (1.09) | 8.52 (1.17) | 6.49 (1.73) |
| 20:2n6 | 3.31 (1.16) | 3.32 (1) | 3.82 (1.1) | 3.09 (0.72) | 3.17 (0.51) |
| 20:3n6 | 0.59 (0.75) | 0.53 (0.22) | 0.56 (0.35) | 0.46 (0.25) | 0.34 (0.15) |
| 20:4n6 | 1.94 (0.69) | 2.4 (0.66) | 2.27 (0.8) | 2.88 (1.24) | 1.67 (0.38) |
| 20:3n3 | 0.62 (0.36) | 0.65 (0.29) | 0.62 (0.17) | 0.53 (0.08) | 0.4 (0.15) |
| 20:4n3 | 0.68 (0.64) | 0.54 (0.46) | 0.5 (0.28) | 0.3 (0.14) | 0.34 (0.15) |
| 20:5n3 | **15.52 (3.53)** | **16.26 (3.51)** | **14.69 (3.33)** | **18.61 (3.87)** | **13.24 (4.19)** |
| 22:0 | 0.06 (0.06) | 0.09 (0.07) | 0.11 (0.08) | 0.14 (0.11) | 0.05 (0.06) |
| 22:1n11 | 2.94 (2.51) | 1.9 (1.17) | 1.88 (1.21) | 1.68 (1.08) | 2.22 (1.48) |
| 22:1n9 | 0.1 (0.15) | 0.09 (0.08) | 0.1 (0.07) | 0.07 (0.08) | 0.1 (0.09) |
| 22:4n6 | 1.52 (0.98) | 1.51 (0.77) | 1.35 (0.84) | 1.96 (0.63) | 0.86 (0.53) |
| 22:5n3 | 2.87 (1.13) | 2.87 (1.15) | 2.3 (0.82) | 2.97 (1.2) | 1.79 (0.67) |
| 22:6n3 | **2.05 (1.67)** | **1.57 (0.69)** | **1.5 (1.01)** | **1.7 (0.9)** | **5.47 (2.58)** |
| saturated FA | 27.89 (3.68) | 27.89 (3.59) | 29.93 (2.52) | 28.78 (1.89) | 24.28 (3.16) |
| monounsaturated FA | 33.23 (5.13) | 32.46 (4.05) | 32.32 (2.77) | 30.9 (2.01) | 29.8 (3.3) |
| polyunsaturated FA | **38.88 (4.78)** | **39.66 (4.44)** | **37.75 (2.9)** | **40.32 (2.69)** | **45.93 (4.65)** |

The similarity in the fatty acid composition of polychaetes fed side stream diets could be explained by the similar profile of saturated fatty acids (41%), monounsaturated fatty acids (43%) and PUFA (16%) of both side streams. Another similarity was the low lipid content of both side streams (4–7% of DM). These results suggested a lipid homeostatic capacity of *H. diversicolor*, based on its biosynthetic competence for trophic upgrading (Kabeya et al., 2020). Thus, dietary effects would be then more evident when a homeostatic threshold is surpassed, for example with lipid and PUFA-rich diets such as fish feed. Similar regulatory patterns have been described by Passarelli et al. (2012), who

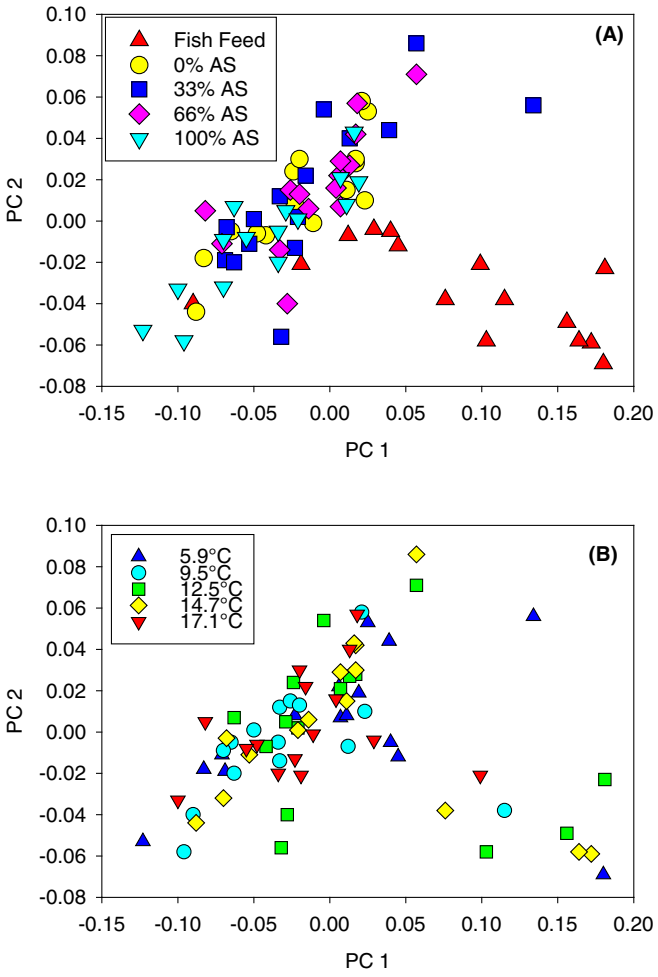


**Fig. 4.** Principal Component Analysis (PCA) on total fatty acids composition of *H. diversicolor* fed different diets at different temperature visualized by (A) feed type (% AS in the diet or fish feed and (B) by the five different rearing temperatures (°C). PC1 = 56%, PC2 = 14%.

reported pronounced differences in several fatty acids between fed and starved *H. diversicolor*, but not in EPA.

Nereid polychaetes can be very effective bacterivore animals (Lopez and Levinton, 1987). *Nereis succinea*, a close relative of *H. diversicolor*, has been shown to assimilate 57% of the available heterotrophic detrital microbes (Cammen, 1980). The enabling mechanism is the bacteriolytic activity of their digestive tract (Scaps, 2002). This activity is carried out by bacteriolytic enzymes such as lysozymes. Bacteriolytic activity may have a dual function in the animal by reducing competition between the consumer and ingested bacteria for organic matter, playing a role in the defense of the polychaete while at the same time providing a further source of nutrients for the animal (Lucas and Bertru, 1997). The presence of typical bacterial fatty acids such as 14:0, 16:0, 17:0 and 18:1n-7 has previously been used to confirm the bacteriolytic activity, not only in *H. diversicolor* (Luis and Passos, 1995), but also in *Arenicola marina* (Grossi et al., 2006) and *Nereis* sp. (Olive et al., 2009). This was also mirrored in the present study, where the capability of digesting bacterial biomass is probably the most important trait of *H. diversicolor* thriving on aquaculture sludge, end even more, on bacteria rich SBD.

## 5. Conclusions

In summary, from a recycling perspective, the results of this study suggest that *H. diversicolor* has a high potential as a candidate for upgrading aquaculture sludge as well as SBD. Further, while not

determining remarkably the fatty acid composition, temperature arises as a key factor enabling optimal polychaete growth rates without compromising the fatty acid composition of the *H. diversicolor* when fed on low nutritional value substrates.

## CRediT authorship contribution statement

**Arne M. Malzahn:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Andrea Villena-Rodríguez:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Óscar Monroig:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Åsmund Johansen:** Investigation, Writing – review & editing. **L. Filipe C. Castro:** Conceptualization, Writing – review & editing. **Juan C. Navarro:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Andreas Hagemann:** Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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