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A new strategy in rearing of European flounder: using live *Enchytraeus albidus* to enhance juvenile growth

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 RESEARCH ARTICLE

Abstract

This study reports the use of the annelid worm *Enchytraeus albidus* as a live feed for reared juvenile European flounder, *Platichthys flesus.* We investigated growth, lipid composition and mortality in small scale (hundreds of fish) and large scale (thousands of fish) rearing. Live *Enchytraeus* had a relatively low content of the essential omega-3 fatty acid, docosahexaenoic acid (DHA), but similar levels of other essential fatty acids and amino acids as the normally used commercial dry feed. Despite low DHA levels in live worms, juvenile flounder fed *Enchytraeus* grew twice as fast as fish fed commercial dry feed. DHA levels in membrane phospholipids of juvenile flounder became depleted when fed *Enchytraeus*, but this had no apparent negative consequences for development or health of the fish. Our study suggests that the use of *Enchytraeus* as a live feed for European flounder is feasible at a large scale.

Keywords: invertebrates, live feed, flatfish, growth performance, fatty acids, aquaculture

1. Introduction

The demand for protein to sustain the world's growing population requires increased and sustainable fish production in the near future. Forecasts suggest that production of aquatic animals will increase by 32% to reach 109 million tonnes in 2030 (FAO, 2020). Hence, aquaculture is a fast-growing animal food production sector, but the needs for high-quality live feed for early life stages of many fish species may be a limiting factor. Almost all marine fish and some freshwater fish, including farmed high-value species, need live feed in the initial life stages. The aquaculture sector is therefore dependent on the supply of good quality live feed in order to ensure fry production for fish farming. This means that new and ecologically sustainable forms of live feed are needed to increase the survival and growth of larval and juvenile stages of fish.

Larviculture of marine fish requires a supply of zooplankton organisms rich in protein and essential fatty acids. Typically, small zooplankton such as rotifers, *Artemia* and copepods are used as the first live feed (Hamre *et al.*, 2013; Støttrup and McEvoy, 2003; Støttrup *et al.*, 1998). Copepods are grazers of microalgae and therefore contain high amounts of essential fatty acids, whereas *Artemia* needs to be enriched with highly unsaturated fatty acids (HUFAs), in particular, arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in order to ensure optimal development of fish larvae (Evjemo and Olsen, 1997; Hamre *et al.*, 2013). In the late larval development and after metamorphosis, the limited supply and small size of the currently used live feeds can be insufficient to sustain high growth rates due to increasing feed demands of the fish larvae. This limitation forces the initiation of weaning to commercial dry feed. The transition from live feed to dry feed is a critical period where limitations in feeding success can cause slow growth rates and increased mortality (Moksness *et al.*, 2004). Therefore, it would be desirable to supplement dry feed with larger live feed organisms of good nutritional quality during this period.

European flounder, *Platichthys flesus*, has been reared for stock enhancement of Danish fjords for more than 30 years. Rearing is based on larval development in semi-extensively managed ponds where the primary live feed consists of copepods (Engell-Sørensen *et al.*, 2004). Soon

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after metamorphosis, the juvenile flounders are moved to indoor tanks, and feeding with commercial dry feed begins, however, growth during the weaning period may be slow. European flounder is not of commercial interest in aquaculture, but we use it here as a surrogate model species for other commercially cultured flatfish species in tests of alternative live feeds during the weaning process.

The oligochaete worm, *Enchytraeus albidus* Henle (hereafter *Enchytraeus*), is a promising live feed candidate to bridge the gap between late feeding in ponds with zooplankton and the onset of feeding dry feed to juvenile flounder. *Enchytraeus* is euryhaline and survives extended periods in both freshwater and seawater, where it will wriggle and stimulate fish to feed on them (Walsh *et al.*, 2015). Production of *Enchytraeus* as live feed has reached a semi-industrial level (Ivleva, 1973; Vedrasco *et al.*, 2002), but more refinement of cultivation techniques is needed that can increase the content of HUFA (Dai *et al.*, 2021; Fairchild *et al.*, 2017). *Enchytraeus* can potentially be grown on organic waste products supporting a circular bioeconomy and environmental sustainability (Fairchild *et al.*, 2017; Ivleva, 1973; Memis *et al.*, 2004). The suitability of *Enchytraeus* as a live feed for juveniles of some freshwater and marine fish species has been documented in earlier studies (Bouguenec, 1992; Ivleva, 1973; Walsh *et al.*, 2015), but has not been investigated for European flounder.

The aim of the present study was to investigate a new approach in production of flounder using *Enchytraeus* as a live feed during the critical weaning period. To this end, we carried out two feeding experiments comparing growth and survival of newly metamorphosed flounder juveniles fed either live *Enchytraeus* or commercial dry feed pellets (traditional practice). The first experiment used small scale aquaria and focused on feed type effects on growth and fatty acid composition of juvenile fish. The second experiment reflected the currently used production of flounder in larger fish tanks using *Enchytraeus* during the weaning period. We show that growth of juvenile European flounder was twice as high when fed *Enchytraeus* compared to traditional dry feed, despite depletion of DHA content of flounder membrane lipids.

2. Materials and methods

Rearing of European flounder juveniles

Adult flounders caught by local fishermen of the Limfjord, Denmark, were stripped and the eggs fertilised at the facilities of Venøsund Fisk og Skaldyr (Struer, Denmark). Newly hatched larvae were transferred to outdoor ponds containing semi-extensively managed phyto- and zooplankton communities supplying the flounder larvae with a suite of feed organisms (mainly rotifers and copepods). Further details of the pond systems and development of flounder larvae have been described by Engell-Sørensen *et al.* (2004). After metamorphosis, the juveniles were caught by slowly dragging a gillnet from one end of a pond to the other and collecting the juveniles in a small area. At this stage, the juveniles were about six weeks old and had a body length of about 15 mm. The juvenile flounders were at random moved to designated indoor tanks for immediate initiation of the feeding trials (either traditional dry feed or live *Enchytraeus*).

Production and harvest of *Enchytraeus*

Enchytraeus were produced at the facilities of Aarhus University (Silkeborg, Denmark). In brief, worms were cultured in a 1:4 (vol:vol) mixture of agricultural sandy loam and dried seaweed (mainly *Fucus* spp.) collected near Aarhus, Denmark. Optimal moisture content was established by thoroughly mixing saltwater (15 ppt salinity) and dry substrate in a ratio of 200 ml saltwater to 1 kg of dry soil/seaweed mixture. The worms were fed oatmeal *ad libitum*. *Enchytraeus* worms were extracted by applying heat to the bottom of culture boxes, which caused worms to migrate to the surface of the soil and the sides of the boxes where worms could be scooped off. This procedure yielded a mixture of different sizes of worms ranging from small juveniles (2-4 mm) up to adults (15-25 mm). *Enchytraeus* worms were rinsed in cool tap water, and then manually distributed between tanks (see later sections).

Small-scale trial in aquaria

We performed a small-scale feeding experiment in June 2019. Six 60 l transparent glass tanks were filled with filtered seawater (salinity 28.5 g/kg), and 50 juvenile flounder were added to each tank. Gentle bubbling in one end of the tank ensured sufficient oxygen levels (>95% dissolved oxygen saturation). The experiment was carried out in constant light, but water temperature during the experiment ranged from 16.4 to 20.3 °C (mean 18.4 °C) due to the changing local weather affecting indoor temperatures. Every second day, 70-80% of the water was exchanged with fresh seawater. Each diet group was represented by three tanks chosen at random and fed for 13 consecutive days with their respective food source. Fish assigned *Enchytraeus* were fed *ad libitum* by manually adding worms three times a day. As worms survived for many days in the tanks, uneaten worms did not compromise water quality to any noticeable extent. Fish assigned dry feed were fed Skretting Perla larva Proactive 5.0 with a particle size of 200-400 μm (Skretting, Stavanger, Norway) *ad libitum*. Uneaten dry feed or worms that had sunk to the bottom was removed during the water exchange every second day.

Trials in fish tanks

The experiment in fish tanks was carried out in June 2020. Six 2 m^3 tanks were filled with filtered seawater (salinity 28-30 g/kg), and 1000 juvenile fish were added. Throughout the experiment, we maintained a continuous flow of fresh seawater (800 l/h), ensuring water exchange and >95% dissolved oxygen saturation. Each diet group was represented by three tanks chosen at random and fed for 14 consecutive days with their respective food source. Water temperature during the experiments ranged from 12.3 to 16.9 °C in continuous light conditions. In this experiment, *Enchytraeus* were manually distributed between tanks three times a day. Half of a tank's *Enchytraeus* were handfed, and the rest placed in a partly submerged cylinder which was closed by a 0.5 mm mesh allowing slow delivery of worms as they crawled through the mesh (Bouguenec, 1992). The fish assigned dry feed were fed Skretting Perla larva Proactive 5.0 *ad libitum* using automated feed dispensers.

Fish growth and mortality

Fish selected for termination and later analysis were euthanised using an overdose of the anaesthetic tricaine methanesulphonate (TMS)(Pharmaq AS, Overhalla, Norway). As a start reference for growth, 35 randomly collected juvenile flounders were euthanised by TMS on the first day of the experiment. Similarly, at the end of the experiment 35-50 randomly chosen juveniles were sampled from each tank, euthanised by TMS treatment, and used for later determination of length, dry weight and lipid composition (only in the small-scale experiment).

Standard length was measured digitally by taking pictures of individual fish placed on a millimetre scale. Fish were then placed individually in Eppendorf tubes and snap-frozen in dry ice for later dry weight and lipid analysis. Dry weight was determined with a precision of 0.01 mg after drying at 80 °C for 48 hours. Fulton's condition factor was calculated using the formula $K = 100\ (W / L^3)$, where W is dry weight and L is length in mm (Nash *et al.*, 2006). A subset of fish from the small-scale experiment was freeze dried and stored at -80 °C until they were used for analysis of lipid composition. Fish from the fish tank experiment were only used for growth and mortality measurements.

Fish mortality was determined based on the sum of daily death counts during the experiment. In the small-scale experiment mortality was counted every second day, and in the fish tank experiment mortality was counted daily. Total mortality was calculated as the number of dead fish divided by the sum of dead and live fish (presented as a percentage).

Chemical analysis

The composition and content of fatty acids of fish and feed were analysed as described by Holmstrup *et al.* (2020) with a few modifications. In brief, total crude lipids were extracted from whole fish or approximately 5 mg freeze-dried material of worms (ca. 10 worms) or dry feed using a modified Bligh-Dyer single-phase method with 2:1:1 v/v/v chloroform:methanol:phosphate buffer in two steps (Waagner *et al.*, 2013). Lipids were re-dissolved in chloroform and vacuum-filtered through solid-phase silica columns (100 mg; Bond Elute, Agilent Technologies, Santa Clara, CA, USA). The lipids were eluted sequentially (non-polar to polar lipids) with chloroform, acetone and finally methanol. The chloroform and acetone fractions contained neutral lipid fatty acids (NLFA), and were pooled before analysis. The methanol fraction contained mainly membrane phospholipid fatty acids (PLFA). The two lipid fractions (NLFA and PLFA) were dried under gentle nitrogen flow. Lipids were then transesterified by a mild alkaline methanolysis to fatty acid methyl esters and subjected to GC-MS identification and quantification as described in detail by Holmstrup *et al.* (2020).

Total amino acid content and composition (of proteins) of worms and dry feed was analysed by an accredited reference laboratory following Danish standards for nutritional composition of food and feed material (Eurofins, Vejen, Denmark). Only one large pooled sample (ca. 10 g) of each feed type was analysed.

Statistical analysis

All statistical analyses were performed in R (Version 4.0.4) (R Core Team, 2019). Data on length, weight, and condition factors were analysed using mixed-effect models fit by REML (restricted maximum likelihood), with Feed type as fixed factor and Tank number as random factor. The analyses were performed using the lme() method in the R packages nlme (Pinheiro *et al.*, 2021). Data were normal or transformed to improve normality and homoscedasticity, which were checked by visual inspection of residual plots. Data on fish mortality were analysed using linear models for fixed effects using the lm() method in R. Contents of selected fatty acids in fish feed and juvenile flounder was subjected to analysis of variance (ANOVA) and posthoc (Holm-Sidak) tests for comparisons of mean values. For all tests, we applied a significance level of $P<0.05$.

3. Results

Nutritional composition of feeds

The content of total protein was 43% of dry weight in *Enchytraeus* and about 57% in dry feed (Table 1). Lipid contents (in fatty acid equivalents) were similar in the two feeds, whereas ash content was somewhat higher in dry feed (Table 1). The amino acid composition of the two feed types were fairly similar, and they had the same proportions of key essential amino acids (Table 1).

On a dry weight basis, *Enchytraeus* and dry feed had almost the same total fatty acid content. The essential fatty acids, linolenic acid (LNA) and EPA, were slightly more abundant in dry feed, whereas DHA was much lower in *Enchytraeus* than in dry feed (P<0.05; Figure 1). AA was much more abundant in *Enchytraeus* than in dry feed (P<0.05). The full compositions of fatty acids are shown in Table S1.

Table 1. Approximate contents of protein, lipid, carbohydrate, ash and composition of amino acids in the protein fraction of *Enchytraeus albidus* worms and commercial dry feed.[1]

	Dry feed	*E. albidus*
Macronutrients (% of dry matter)		
Crude protein	57.3[a]	42.8[b]
Lipids	11[a]	13.3[b]
Glycogen/starch	nd	19.4[b]
Ash content	8[a]	2.3[b]
Key limiting essential amino acids (% of total protein mass)		
Lysine	6.3	7.2
Methionine	2.3	2.6
Threonine	4.1	5.4
Arginine	5.7	6.0
Tryptophan	1.1	1.4
Other amino acids		
Isoleucine	4.2	4.8
Leucine	7.6	7.9
Histidine	2.4	2.5
Phenylalanine	4.5	4.5
Tyrosine	3.3	3.7
Valine	4.9	5.4
Alanine	5.7	7.4
Asparagine	8.1	8.9
Glutamine	20.1	13.9
Glycine	6.1	5.6
Hydroxy proline	0.7	0.0
Ornithine	0.2	0.8
Proline	6.9	4.9
Serine	4.6	5.6
Cysteine+cystine	1.4	1.6

[1] [a] refers to values from Skretting.com; [b] to values from Dai *et al.* (2021); nd = not determined.

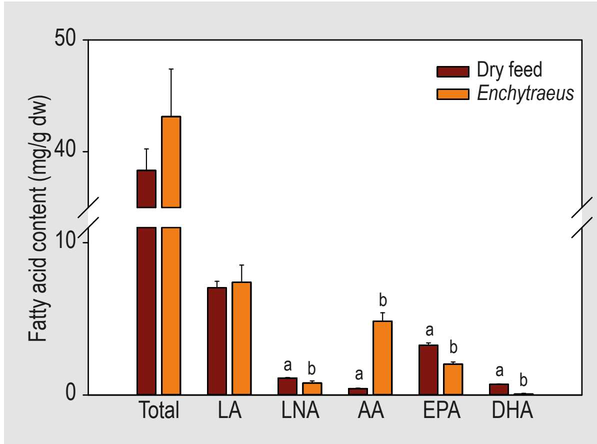

Figure 1. Total lipid content and contents of selected essential fatty acids in commercial dry feed and *Enchytraeus albidus* used in the fish trials. Error bars show standard deviation (n=3 for *Enchytraeus* and n=5 for dry feed). Different letters above bars indicate significant differences (Holm-Sidak, *P*<0.05). AA = arachidonic acid (20:4ω6); DHA = docosahexaenoic acid (22:6ω3); EPA = eicosapentaenoic acid (20:5ω3); LA = linoleic acid (18:2ω6); LNA = linolenic acid (18:3ω3).

Growth and mortality of flounder

Feeding with both dry feed and *Enchytraeus* for two weeks resulted in increased length and body mass (Figure 2A and 2B). Feeding with *Enchytraeus* in the small-scale experiment gave significantly higher increase in average standard length, dry weight and condition factor than feeding with dry feed (Figure 2; standard length: $F_{1,4}$=55.14, P=0.002; dry weight: $F_{1,4}$=106.05, P<0.001; K: $F_{1,4}$=118.61, P<0.001).

In the fish tank experiment, we saw similar results in growth parameters, although more significant. Feeding with *Enchytraeus* resulted in even higher increases in growth parameters as compared to feeding with dry feed (standard length: $F_{1,4}$=222.39, P<0.001; dry weight: $F_{1,4}$=171.93, P<0.001; K: $F_{1,4}$=183.35, P<0.001). Notably, fish fed *Enchytraeus* increased their dry weight almost 4-fold, whereas the fish fed dry feed doubled their dry weight. Data from the growth experiment at tank level are shown in Table S2.

In the small-scale feeding trial, we observed that average mortality (mean ± SD, n=3) within *Enchytraeus* and dry feed tanks after 13 days were 5.6±8.7 and 11.3±6.1%, respectively. Even though the average mortality of fish given dry feed was double that of *Enchytraeus* fed fish, this difference was not statistically significant ($F_{1,4}$=0.844, P=0.410). In the fish tank feeding trial, the average mortality in fish fed *Enchytraeus* and dry feed was similar and low (0.4±0.3 and 0.3±0.2%, respectively, $F_{1,4}$=0.1, P=0.678). Tank specific average mortality values are shown in Table S2.

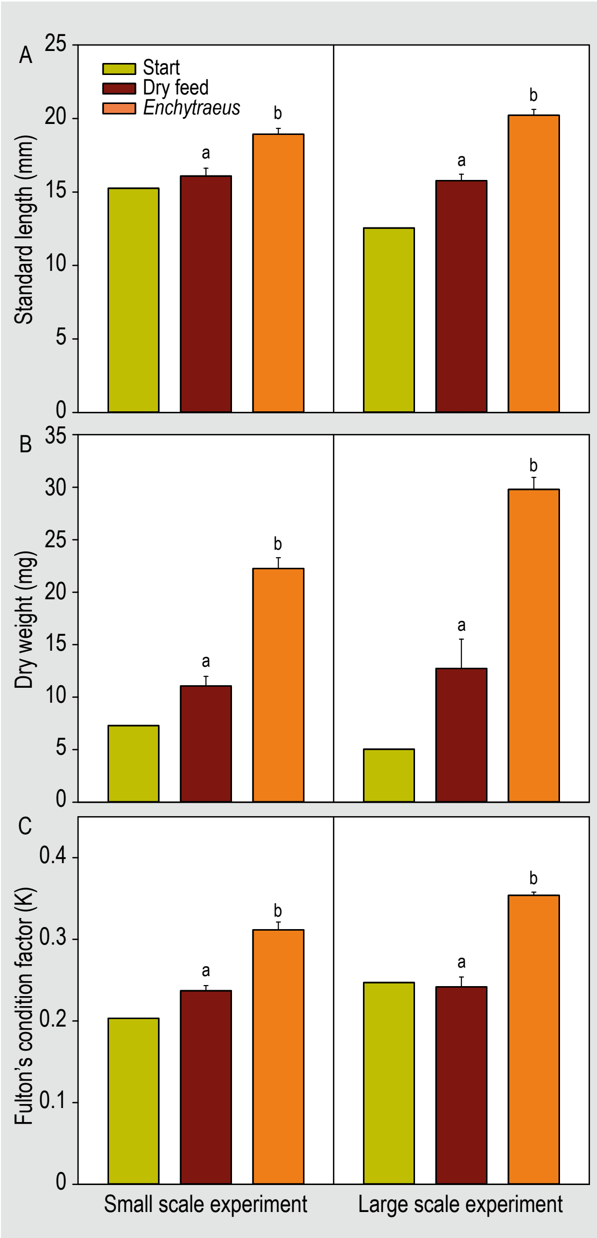

Figure 2. (A) standard length (mm), (B) dry weight (mg), and (C) Fulton's condition factor (K), obtained from small- and large-scale experiment comparisons between fish fed with traditional dry feed and *Enchytraeus albidus* live feed. Error bars show standard deviation (n=3) and different letters above bars indicate significant differences between feed types (Holm-Sidak, *P*<0.05). Note that mean values at the outset of the experiment ('Start') are shown for illustration, but are not included in the statistical comparison.

Fatty acid composition of fish

Feeding juvenile flounder with dry feed for two weeks resulted in an increased proportion of LA in membrane phospholipids, but the proportions of LNA, AA, EPA and DHA did not change (Figure 3). It could be argued that our choice of dry feed product was not optimal for flounder, however, our analysis shows that the EPA and DHA levels in fish PLFAs did not change in the dry feed treatment despite substantial growth of the fry (so no dilution of these fatty acids occurred). Hence, we suggest that the used fry feed was not sub-optimal with regard to fatty acid composition. Feeding with *Enchytraeus* had larger effects on membrane phospholipids significantly increasing proportions of LA and AA, but decreasing EPA and DHA (Figure 3). The full composition of flounder membrane fatty acids is shown in Table S3.

The level of NLFA ('storage lipids') in the start group was very low (below 1 mg/g dry weight), but increased slightly during the experiment for both feed types. Fish fed *Enchytraeus* had a higher total NLFA content than fish fed dry feed (*P*<0.05; Figure 4). The composition of essential fatty acids in the storage lipids of both feeding groups reflected the composition found in the feed. Thus, AA was highest in *Enchytraeus* fed fish and DHA was highest in fish fed dry feed (*P*<0.05; Figure 4).

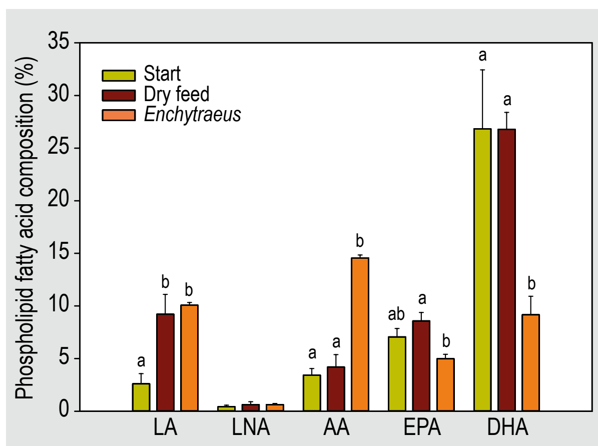

Figure 3. The proportions of membrane phospholipid fatty acids of juvenile flounder at the outset of the feeding trial and after two weeks of feeding with either *Enchytraeus* or commercial dry feed. Error bars show standard deviation (n=6). Different letters above bars indicate significant differences (Holm-Sidak, *P*<0.05). AA = arachidonic acid (20:4ω6); DHA = docosahexaenoic acid (22:6ω3); EPA = eicosapentaenoic acid (20:5ω3); LA = linoleic acid (18:2ω6); LNA = linolenic acid (18:3ω3).

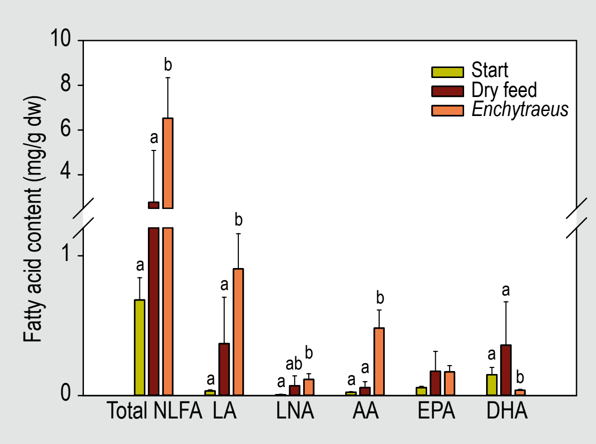

Figure 4. The content of neutral lipid fatty acids in flounder at the outset of the feeding trial and after two weeks of feeding with either *Enchytraeus* or dry feed. Error bars show standard deviation (n=6). Different letters above bars indicate significant differences (Holm-Sidak, $P<0.05$). AA = arachidonic acid (20:4ω6); DHA = docosahexaenoic acid (22:6ω3); EPA = eicosapentaenoic acid (20:5ω3); LA = linoleic acid (18:2ω6); LNA = linolenic acid (18:3ω3).

4. Discussion

Our study is the first to investigate the use of *Enchytraeus* as live feed for recently metamorphosed juvenile European flounders and provides proof-of-concept for this rearing strategy. The main finding of our study was that juvenile flounder grew twice as quickly on *Enchytraeus* as compared to traditional rearing on commercial dry feed, and juvenile fish survived equally well on both feed types. These results are in line with a study by Walsh *et al.* (2015) who found that winter flounder (*Pseudopleuronectes americanus*) grew twice as fast on live *Enchytraeus* compared with commercial pellet feed.

The juvenile flounder used in our study were ca. 12-15 mm long and had, according to Engell-Sørensen *et al.* (2004), a mouth diameter of at least 0.7 mm enabling these fish to ingest relatively large prey. In accordance with this, we observed that the fish readily ingested the soft-bodied *Enchytraeus* worms with a diameter of 0.3-1.0 mm (data not shown). Although we did not perform systematic monitoring of feeding behaviour, our observations indicated that all fish started feeding shortly after (0-25 h) the introduction of *Enchytraeus* to tanks. The fish would often attack and eat multiple worms during a feeding event and exhibited natural feeding behaviours such as flipping to whirl up food items from the bottom. It appeared that the flounders in size 1-2 cm would prefer smaller sized *Enchytraeus* (0.1-0.3 mm diameter) in the first day or two and later also would eat larger worms.

Chemical analysis of *Enchytraeus* and the commercial dry feed revealed that protein content of the dry feed (57% of dry weight) was higher than in worms (43%), but the amino acid profile was almost equal and key limiting amino acids were abundant in both. Total lipid content was also largely equal, but dry feed had significantly higher levels of DHA than in *Enchytraeus*. The low level of DHA in *Enchytraeus* was clearly reflected in the PLFA profile of flounder after two weeks of feeding, but, despite this, the *Enchytraeus* fed fish grew much better than fish given dry feed.

The essential HUFAs such as EPA and DHA cannot be synthesised de novo by marine fish (Berge and Barnathan, 2005; Reis *et al.*, 2021; Sargent *et al.*, 1997). Therefore, successful development is highly dependent on the quality and content of lipids and fatty acids in their live feeds from the onset of exogenous feeding (Sargent *et al.*, 1999). Studies on optimal dietary lipid composition in the feed of flatfish larvae vary with species, life stage and environment (Estevez *et al.*, 1999; Hamre and Harboe, 2008). The dietary requirements dictate the presence of both DHA and EPA, with DHA being more important to fish larvae due to the rapid development of vision and nervous system (Furuita *et al.*, 1998; Watanabe and Kiron, 1994). Not only the levels of EPA and DHA, but also the DHA/EPA ratio has been emphasised as a key factor. With minimum DHA and EPA levels being met, DHA/EPA ratio seems more important for improvement of larval growth and development (Bell *et al.*, 1985; Estevez *et al.*, 1999; Sargent *et al.*, 1999).

Dietary DHA and EPA requirements have been thoroughly investigated for many flatfish species. When compared to other marine fish larvae, dietary DHA and EPA minimum requirements for flounder species such as plaice, Japanese flounder and common sole are generally low (Choi *et al.*, 2014; Dickey-Collas and Geffen, 1992; Furuita *et al.*, 1998, 1999; Izquierdo *et al.*, 1992). This could explain why *Enchytraeus* with relatively low levels of DHA, but EPA levels comparable to dry feed, seemed to be an adequate feed for European flounder. Other HUFAs such as AA may play an important role in successful larval development (Boglino *et al.*, 2014; Copeman *et al.*, 2002; Lund *et al.*, 2007), and also for these HUFAs, the levels in *Enchytraeus* were just as high or higher than in the commercial dry feed. Despite the thorough research done on larval dietary requirements of flounder species we still do not know the exact requirements for most flatfish species (Hamre *et al.*, 2013). To our knowledge, the importance of dietary DHA, EPA and AA to European flounder larval growth and development has not been investigated. It should be noted, however, that most research has focused on growth and development of the larval stages of fish, whereas our study used juvenile fish that has just gone through metamorphosis. It is likely that HUFA requirements are much more important in the early larval stages of flounder where DHA-rich copepods constituted the main food source, and may be less critical in the further growth and development of metamorphosed juveniles. Although the role of fatty acid composition in feed is important, other significant nutritional factors (e.g. vitamin content) are not known in the present study, and further work is therefore needed before we may conclude on which nutrients can explain the differences in growth observed.

Enchytraeus worms differ from many other live feed organisms due to their high content of glycogen, which is typically in the range 15-25% of dry weight (Dai *et al.*, 2021; Fisker *et al.*, 2014). While glycogen is an excellent metabolic fuel, feeding on *Enchytraeus* could potentially lead to obesity. Anecdotal evidence has often claimed that ornamental fish may become too fat if fed *Enchytraeus* worms too often. We investigated this by analysing the storage lipid (NLFA) content of juvenile flounder. We found no evidence of obesity even though NLFA levels were slightly higher in fish fed *Enchytraeus*.

By feeding with live *Enchytraeus* worms we have here shown that growth rates in flounder fry production can be substantially better as compared to the common practice where commercial dry feed is used. Lastly, it should be mentioned that feeding with live feed can be used as a pre-conditioning of fry to a more natural foraging behaviour. Several studies have indicated that acclimating hatchery-reared fish to natural-type food items can improve foraging efficiency when fish fry is released in restocking programs as has been shown for turbot fry (*Psetta maxima*) and various salmonids (Brown and Day, 2002; Ellis *et al.*, 2002).

However, the production of *Enchytraeus* is currently costly and perhaps not economically competitive to commercial dry feed. Supply of *Enchytraeus* for 3,000 juvenile flounder during two weeks required roughly the work effort of one person for a month. This cost included maintenance of culture boxes and daily harvest and distribution of *Enchytraeus* during the fish trial. Costs of consumables used for *Enchytraeus* production, however, are negligible. At a first glance, using dry feed would therefore seem easier and economically more attractive. However, if the on-grow period of flounder fry before they are released in stock-enhancement programs could be halved, which our results suggest, a substantial work-effort and consumption of electrical power (for water treatment, lighting, etc.) could be saved. More experimental work and a detailed life cycle analysis is therefore needed to judge the feasibility of using *Enchytraeus* as a live feed, not only for European flounder, but also for other fishes where there is a need to bridge the gap between live feed and dry feed in marine fish larvae production.

In conclusion, we observed that juvenile flounder fed *Enchytraeus* grew twice as fast as fish fed commercial dry feed despite the low DHA levels of live worms. DHA levels in membrane phospholipids of juvenile flounder did become depleted when fed *Enchytraeus*, but this had no apparent negative consequences for development or health of the fish. Our study suggests that the use of *Enchytraeus* as a live feed for European flounder is feasible at a large scale.

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Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2021.0106

Table S1. The full composition of fatty acids in commercial dry feed and *Enchytraeus albidus* worms.

Table S2. Mean and standard deviation of standard length, body dry mass and Fulton's condition factor (K) in the small-scale (A) and large-scale (B) fish trials.

Table S3. The full composition of membrane phospholipid fatty acids of juvenile flounder at the outset of the experiment ('Start'), after two weeks of feeding with commercial dry feed and after two weeks feeding with *Enchytraeus*.

Conflict of interest

MH and KES are married and economically dependent. KES is the owner of Fishlab, and plans to produce *Enchytraeus* worms on a commercial basis. No other conflicts of interest are declared.

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