Journal of Experimental Marine Biology and Ecology 381 (2009) 82–89

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Deep-water shrimp (*Pandalus borealis*, Krøyer 1838) as indicator organism for fish-farm waste

Siri Aaserud Olsen [a], Arne Ervik [a], Otto Grahl-Nielsen [b,*]

[a] *Institute of Marine Research, PO Box 1870, Nordnes, N-5817 Bergen, Norway*
[b] *Department of Chemistry, University of Bergen, N-5007 Bergen, Norway*

ARTICLE INFO

Article history:
Received 29 January 2009
Received in revised form 28 September 2009
Accepted 28 September 2009

Keywords:
Fatty acid composition
Feeding experiment
Fish-farm waste
Lipid biomarker
Pandalus borealis
Tracers

ABSTRACT

A feeding experiment was carried out with deep-water shrimp (*Pandalus borealis*), a common benthic inhabitant of Norwegian fjords. Shrimp were reared in tanks for three months, fed either salmon feed or cod, and the fatty acid profile of their muscle tissue was monitored. The salmon feed pellets and cod had completely different fatty acid compositions, with significant differences in most of the analysed fatty acids, eight of them, 14:0, iso16:0, 16:1n7, 16:2n11, 18:3n3, 20:0, 22:0 and 22:1n11, differing between the two diets by a factor of 10 or more. The levels of the fatty acids in the diets differed also substantially from the levels in the shrimp tissue at the start of the experiment. The shrimp were fed *ad libitum* and both groups increased significantly in total fatty acid content in the course of the experiment. Nevertheless, the fatty acids made up no more than 6.5 mg g^{-1} muscle tissue, with 88 ± 1% of them bound in polar, membrane lipids. Their composition in the tissue did also change to a certain extent, but the resulting tissue composition did not reflect the fatty acid composition of the diets. Only two fatty acids, 18:2n6 and 18:3n3, clearly followed the dietary level. They are abundant in commercial salmon feed since they are rich in plant oils used as additives to the commercial feed. Determination of fatty acid levels in shrimp tissue may enable shrimp to be used as indicator organisms for the influence of organic fish-farm waste on the food web in adjacent areas.

1. Introduction

Norwegian production of Atlantic salmon (*Salmo salar*) is currently mainly carried out in large-scale fish farms at deep-water locations (>100 m) with good water exchange. Over-exploitation of fish-farm sites is therefore no longer a general problem despite a production rate of 3000–5000 tons per 18-month cycle on a typical farm site and a level of feed consumption that may reach 15–20 tons per day. However, fish farms still have a great influence on benthic fauna and may cause ecological changes such as increased abundance, biomass and production (Tvedten et al., 1995; Johansen et al., 2001; Kutti et al., 2007a) through the large amounts of organic material discharged in the form of faeces and excess feed. The organic waste may be dispersed to large areas via a resuspension process (Stigebrandt et al., 2004; Kutti et al., 2007b) and the total ecological impact of the approximately 1000 Norwegian fish farms must be regarded as substantial.

If we are to understand and quantify these ecological impacts, we must be able to trace and quantify the organic material through the trophic levels. Analyses of fatty acid (FA) composition have been used in several feed experiments with fish (Kirsch et al., 1998; Bell et al., 2003a,b), trophic interaction studies (Graeve et al., 1994 Graeve et al., 1997; Cripps and Atkinson, 2000; Stubing et al., 2003) and to trace organic material origin in different ecosystems (Meziane et al., 1997; Dalsgaard et al., 2003). FA composition has proved to be an efficient tracer for the distribution of organic waste in sediment beneath fish farms (Samuelsen et al., 1988; Johnsen et al., 1993; Henderson et al., 1997) and FAs that are typical of fish feed have been recognized in organisms feeding close to fish-farm sites (Skog et al., 2003; Cook et al., 2000; Gao et al., 2006).

Previously, salmon feed was mainly based on fishmeal and fish oil. This has now changed and today approximately 30% of the fats in salmon feed are of vegetable origin (Skretting, 2005) e.g. soybean, rapeseed and linseed. These plant oils contain high concentrations of, for example, FAs 18:2n6, 18:3n3 and 18:1n9 (Gunstone et al., 1994); Table 1. Marine fat mainly consists of long-chained polyunsaturated FA (PUFA), especially 20:5n3 and 22:6n3 (Morris and Culkin, 1976), which are found in high concentrations in both fishmeal and fish oil. These products are also enriched in other FAs that are typical of marine sources, such as 22:1n11 and 20:1n9, which are found in high concentrations in capelin and herring, for example (Budge et al., 2002). Commercial fish feeds based on these different ingredients have a FA composition that differs from that of the biota at the locations of the fish farms. The total FA pattern or selected FAs may therefore be used as markers for organic waste from the farms.

Deep-water shrimp *Pandalus borealis* (Krøyer, 1838) are found in great numbers in Norwegian fjord systems at depths of 100–250 m

---

* Corresponding author: Tel.: +47 55583553; fax: +47 55589490.
E-mail address: Otto.Grahl-Nielsen@kj.uib.no (O. Grahl-Nielsen).

0022-0981/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2009.09.019

Table 1
Relative amounts, as percentage of total FA, of the most abundant FA in various plant oils used in production of salmon feed (Gunstone et al. 1994).

Fatty acid	Soybean oil	Rapeseed oil	Linseed oil	Palm oil
18:1n9	18–26	52–67	15–23	36–44
18:2n6	50–57	16–25	15–18	6–12
18:3n3	6–10	6–14	50–60	0–0.5

(Hjort and Ruud, 1938). Shrimp make vertical migrations and feed both pelagically and benthically. Adult individuals have a basically benthic diet consisting of polychaetes, carcases and substrate while younger individuals have a more pelagic diet based on zooplankton such as copepods and euphausiids (Hopkins et al., 1993). Shrimp is an economically important species in Norwegian fisheries. There have been some conflicts between fish farmers and shrimp trawlers, as the trawlers claim that the quality and catches of shrimp decrease in the vicinity of farms. However, no studies confirm whether shrimp utilize organic waste from fish farms and how such utilization might influence their chemical composition.

We have carried out a feeding experiment on *P. borealis* to study how FAs from typical feed pellets used in salmon farming are laid down in the lipids in the muscle tissue. Cod muscle was used as reference feed. Being the first feeding experiment ever performed using adult *P. borealis*, the purpose was to see if the FA pattern of the tissue lipids reflects the FA pattern of the diet in general, and furthermore to find out whether the whole pattern, or selected FAs, in the shrimp muscle can be used as markers for the waste from a fish farm.

2. Materials and methods

2.1. Collection of material

Deep-water shrimp were collected in January 2004 from Fanafjorden 15 km south of Bergen at depths of 135–150 m, using a 20 mm mesh sized shrimp trawl. There has been no fish-farm activity in the fjord and the nearest fish farms are located 7–12 km from the trawling site. About 1000 shrimp were put in well-flushed tubs and transported to tanks at the Institute of Marine Research (IMR) in Bergen.

2.2. Experimental design

The feed experiment took place at IMR from February to May 2004. The shrimp were acclimatised for two weeks without food, and then 80 individuals were transferred to each of six tanks. The tanks were 160–400 l, had a gravel substrate and a 12-hour light/dark regime. The water supply was a mix of seawater from inlets at depths of 50 and 150 m in the fjord outside IMR. The flow into the tanks was 6–7 L min^{-1}, the temperature was 8 °C and the salinity varied between 32‰ and 34‰. The tanks were divided into two feed groups. The ‘Pellet group’ was fed commercially available salmon feed pellets (EWOS, Pyramid Exp 2500; 10.5 mm). The ‘Fish group’ was fed cod filet from Bergen Aquarium reared on a diet based on squid and herring, and therefore not expected to contain any components derived from fish-farm waste.

The shrimp were fed *ad libitum* and the food was replaced every three or four days. Before feeding, uneaten food was removed and the substrate was cleaned by simple suction device. Observations showed that the shrimp ate the accessible food and that they all displayed the same behaviour. Due to technical problems with the water supply on day 53, only 4 and 20 shrimp survived in tanks 5 and 6, respectively.

Samples were collected six times during the experiment, when seven shrimp from each of the six tanks were individually marked and frozen. As a reference material, 20 shrimp were sampled on day 0 before the feeding started.

2.3. Sample preparation

Samples of 30 to 50 mg muscle tissue from the dorsal abdomen were weighed precisely and placed in thick-walled glass tubes containing 19.2 µg of the internal standard FA 19:0. One-half mL anhydrous methanol, containing hydrogen chloride (HCl) at a concentration of 2 mol L^{-1} was added, the tubes were sealed with Teflon-lined screw caps and subjected to methanolysis for 2 h at 90 °C (Meier et al., 2006). After cooling, approximately half of the methanol was evaporated with N_2 gas and replaced by 0.5 mL distilled water. The water/methanol phase containing the FA methyl esters (FAME) was extracted twice with 1 mL hexane by thorough shaking followed by centrifugation and withdrawal of the hexane phase by a Pasteur pipette. The concentration of the FAME in the combined extracts was adjusted by addition of hexane to obtain levels suitable for gas chromatography. Only one sample from each shrimp was analysed, but to control the analytic accuracy four parallels were taken from one randomly selected individual each time samples were collected.

The lipids in the muscle tissue of seven shrimp were individually fractionated into major lipid classes according to the method of Kaluzny et al. (1985) by first extracting them with 10 mL chloroform/methanol, 2:1. The tissue was thoroughly mixed with the solvent using a glass rod and left in the freezer overnight. The solvent was then filtered into a tarred vial and evaporated with N_2 gas to constant weight. The residual lipid was dissolved in chloroform to a concentration of 8 mg mL^{-1}, and 0.5 mL of this solution was applied to an aminopropyl SPE-column (Supelco 500 mg/3 mL), which had been pre-washed with 2×2 mL hexane. The neutral lipid fraction was then eluted with 4 mL chloroform/2-propanol (2/1, v/v). The free FAs were then eluted with 4 mL of 2% acetic acid in diethyl ether. Finally, the polar fraction, i.e. the phospholipids, was eluted with 4 mL methanol. The fractions were collected in thick-walled 15 mL glass tubes, to which the internal standard 19:0 had been added. The solvent was evaporated with N_2 gas, and methanolysis followed by extraction was carried out as described above.

2.4. Gas chromatography

One µL of the hexane extracts was injected splitless and chromatographed on a 25 m×0.25 mm fused-silica column with polyethyleneglycol (CP-WAX 52 CB Chrompack) with 0.2 µm thickness as stationary phase and helium at 20 psi as mobile phase. The column was mounted in a Hewlett–Packard 5892 Series II gas chromatograph equipped with a Hewlett–Packard 7673 autosampler and a flame-ionisation detector. Injector and detector temperatures were 260 °C and 330 °C, respectively. The injection was splitless and the split was opened after 4 min. The oven temperature was 90 °C at injection and this temperature was maintained for 4 min before being raised to 165 °C at 30 °C min^{-1}, thereafter being raised to 225 °C at 3 °C min^{-1} and maintained there for 10.5 min. A standard solution (GLC-68D from Nu-Check-Prep, Elysian, Minn., USA) containing 20 FAME as well as a blank hexane sample was chromatographed after every eight samples. The detector signal was digitalised and sent to Atlas software (Thermo Labsystems). Peaks were identified by means of the standard mixture, previous experience of relative retention times of FAME and mass spectrometry. The peak areas of 26 selected FAME between 14:0 and 24:1n9 were integrated and corrected by response factors. These empirical response factors, relative to 18:0, were calculated for the 20 FAME, present in known amounts in the standard mixture. The average of 10 runs of the standard mixture was used for these calculations. The response factors for each of the six FAME for which we had no standards were estimated by comparison with the standard FAME with the closest retention time that resembled each of those most closely in terms of chain length and number of double bonds. These corrected areas were then used to