Journal of Experimental Marine Biology and Ecology 386 (2010) 61–68

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Unraveling diet and feeding histories of fish using fatty acids as natural tracers

Travis S. Elsdon *

Southern Seas Ecology Laboratory, School of Earth and Environmental Sciences, DX 065 418, University of Adelaide, Adelaide, South Australia 5005, Australia

ARTICLE INFO

Article history:
Received 20 October 2009
Received in revised form 3 February 2010
Accepted 5 February 2010

Keywords:
Acanthopagrus butcheri
Diet
Fatty acid
Nutritional status
Temperature
Turnover

ABSTRACT

Unraveling feeding histories of fish is important if ecologists are to describe the benefits obtained from habitats. I tested if fatty acid (FA) mass and percentage composition can describe fish diet, in the presence of competing factors of feeding duration and temperature. To determine the relative effects of diet, feeding duration (representing temporal dynamics of tissue turnover), and temperature (representing metabolic influence) on fish FA composition, fish were fed two diets that differed in FA composition, protein, and fats and reared at two different temperatures (16 and 23 °C) for different feeding durations (7 and 42 days). Fatty acid composition (total FA matrix) of fish differed with manipulations of diet (FA data as %) and feeding durations (FA data as weights), but not with temperature. Saturated, monounsaturated (MUFA), and polyunsaturated (PUFA) FAs also showed predictable influences of diet and feeding duration, and saturated FA was influenced by an interaction of diet and temperature. The independent effect of diet and feeding duration suggest that FAs are a reliable tracer of dietary history (composition) and also the duration of feeding on different diets. Where differences in diet exist between habitats, the data suggest that FAs may be used to express movement to and from a habitat based.

1. Introduction

It is widely accepted that some individuals or groups of fishes have enhanced survival and success in contributing to consecutive generations depending on where they have lived (Lankford and Targett, 1994; Olla et al., 1998; Fuiman and Cowan, 2003). Benefits fish obtain from different habitats can include dietary constituents that may aid in growth, development, and survival. We need to develop natural tracers of fish diet and feeding histories to help describe which environments sustain fish populations (Policansky and Magnuson, 1998; Gillanders, 2002; Jones et al., 2005). Evidence of diet flow in food-webs can be obtained from estimates of predation and consumption (gut contents etc), and chemical tracers of stable isotopes and fatty acids (FAs). Stable isotopes of carbon and nitrogen can provide qualitative data on food-web positioning and potential dietary sources. The transfer of isotopes is, however, non conservative and involves fractionation. Therefore deciphering the contribution of food to a consumer diet is easy in systems with relatively few food sources (i.e., one or two species of primary producer/consumer), but becomes increasingly difficult in complex food-webs (Fry, 2006). Similarly, food sources that are high in carbohydrates as opposed to protein may be under-represented in reconstructions of diet (Focken and Becker, 1998; Schlechtriem et al., 2004). FAs potentially overcome these issues, because individual FAs are generally conservatively passed in the food-web.

Fatty acids are essential dietary components for fish, as they are precursors to hormones related to growth and development (Sargent et al., 1999). However, the majority of FAs are not synthesised by fish, but usually must be obtained from dietary sources. In this instance, FAs can act as biochemical indicators of food-webs (Wilson et al., 2001; Dalsgaard et al., 2003) as primary producers synthesise unique FAs *de novo* that theoretically are conservatively passed on to, and can be detected in, higher trophic levels (Dalsgaard and St John, 2004). The conservative nature of FAs, particularly polyunsaturated FA (PUFA) which are incorporated from prey, suggests that FAs can act as reliable indicators of diet and the feeding duration on different diets, and as such, FAs can address questions on habitat use and origin (e.g., movements into habitats) (Stowasser et al., 2006; Haas et al., 2009).

Although the use of FAs to determine dietary links is not new, their validation as natural tags to trace diet in the face of other influencing factors requires further research. Several factors are likely to influence the conservative transfer of FAs from diet to fish, including abiotic influences of temperature and biotic factors of tissue turnover leading to turnover of FAs. There are, of course, many other biological and biochemical factors that influence FA signatures, including nutritional state of organisms, uptake efficiencies of food, routing of carbon to different tissues, and growth (Dalsgaard et al., 2003), however, many of these factors also co-vary with the diet and temperature. Temperature affects both fish metabolism and feed conversion (Caulton, 1978), both of which may influence the turnover rate of FAs within muscle of fish. The interaction of temperature and diet

* Tel.: +61 8 8303 7036; fax: +61 8 8303 4364.
E-mail address: travis.elsdon@gmail.com.

0022-0981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2010.02.004

could cause FAs to reflect diet in a non conservative manner. Turnover rate of FAs will be influenced by the duration of time fish have fed on dietary items, as has been detected for stable isotopes (e.g., Bosley et al., 2002; Cuelinckx et al., 2007). Food items consumed by a fish would be incorporated over time rather than instantly, and diets consumed for a short periods, such as bursts of terrestrial insects in systems due to annual hatching, will be under-represented in the FA tissue composition of consumer fish compared to long-term dietary items, such as macrophytes. Such effects have been described for stable isotopes (e.g., Herzka and Holt, 2000; Logan et al., 2006), and would allow for the description of movement to and from environments based on switches in diet and turnover rates of FAs within fish in a similar manner to stable isotope and otoliths (Elsdon and Gillanders, 2003; Elsdon et al., 2008).

To deduce diet of fish using FAs it is important to understand how abiotic and biotic factors influence consumer FAs. I experimentally manipulated diet, temperature, and feeding duration, to determine the effect these would have on FA composition of fish. I hypothesised that effects of diet and feeding duration would have large effects on FA composition, but that any effects may be modified by temperature. The presence of temperature effects would indicate a metabolic effect on FA assimilation from diet to consumer.

2. Materials and methods

The experiment was done on juvenile black bream (*Acanthopagrus butcheri*, Munro 1949), an omnivorous estuarine species common throughout temperate Australia. Black bream (~500) were sourced from hatchery broodstock to reduce genetic variation and standardise initial size differences (~25 mm long). Black bream were transported to the University of Adelaide where they were placed in 1000 L acclimation tanks, and fed twice daily to satiation on a commercial diet prior to allocation into experimental treatment tanks. Acclimation tanks were kept at a constant ambient temperature of 16 °C, and water was changed every other day. Fish were divided into treatment tanks (6–10 fish per tank) that contained filtered seawater with ambient salinities of 40 ppt. Treatments consisted of orthogonal combinations of low and high temperatures (16 and 23 °C), two diets (fish-based and vegetable-based), and two feeding durations (7 and 42 days). Temperatures were chosen to represent average winter and summer conditions within local estuaries (Elsdon et al., 2009), and are within the thermal tolerance of this species (Elsdon and Gillanders, 2002; Norriss et al., 2002). Diets were both commercial fish pellets that differed in their protein sources: fish-based protein and vegetable-based protein, which had different FA composition (Fig. 1) and therefore food quality. Diets differed in composition of protein, fat, and fibre. The vegetable-based diet contained 28, 4, and 7% (protein, fat, fibre) while the fish-based diet contained 43, 9, and 2% (protein, fat, fibre) and the initial hatchery diet contained 45 and 22% (protein and fat, fibre content unavailable). Feeding duration was manipulated to examine the effect of tissue turnover (temporal dynamics) on FA composition. To manipulate feeding duration, fish were sacrificed after rearing in treatments for 7 or 42 days. Each treatment consisted of duplicate tanks, to maintain a fully orthogonal design with three factors (2 temperatures × 2 diets × 2 feeding durations × 2 replicate tanks of each).

Fish tissue was dissected immediately after they were sacrificed and white muscle samples were collected and freeze dried for 72 h ($n = 5$ fish per tank). Muscle samples from each individual fish were homogenized after freeze drying using a mortar and pestle, and 20 mg of this homogenate was placed in a clean microcentrifuge tube awaiting analysis. Diets were also freeze dried ($n = 3$ per diet) then homogenized and 20 mg placed in a clean microcentrifuge tube.

Fatty acids of fish and diets were determined using direct methylation and FA methyl esters were determined by gas chromatography (GC). All samples (fish and diet) were analysed the same way, which included a transesterification solution being added to each sample, which comprised of methoanol:hydrochloric acid:chloroform (10:1:1 per volume). Samples were suspended in this solution by vortex and transesterification occurred at 90 °C, after which samples were cooled and FA methyl esters extracted in triplicate using hexane: chloroform (4:1 per volume)(Lewis et al., 2000). Samples were made up to a known volume using standards and analysed by GC using an Agilent 6890N GC. Peaks were quantified using Agilent GC GhemStation software, and peaks were used to determine relative proportions of FAs (Mooney et al., 2007). A total of 43 FAs were quantified, 12 of these were saturated FA, 14 were monounsaturated FA (MUFA), and the remaining 17 were polyunsaturated FA (PUFA), two of which had undifferentiated carbon chains (C16 and C24 undifferentiated) (Table 1). Results typically had error of ±5% for each FA. FAs were analysed as FA weight (μg FA mg^{-1} dry weight) and also as a percentage, such that individual FA or groups of FAs (saturated FA, MUFA, and PUFA) were converted to a percentage of the total FA. This was done because some FA may represent a greater or less portion of the overall FA composition expressed as a percentage rather than as a weight. For fish, FAs data were reported as μg FA mg^{-1} sample dry weight, and also as a percentage of total FA. For diets, data were reported only as μg FA mg^{-1} sample dry weight.

A permutational MANOVA (PERMANOVA) (Anderson, 2001) was used to compare FA signatures among diet, temperature, and feeding duration treatments. Diet and temperature were treated as fixed and orthogonal treatments, with feeding duration considered random and orthogonal, and tanks were nested and random. Data were square-root transformed, resembled using Bray Curtis similarity distance matrices, and permutations were done as residuals under the reduced model. Post-hoc comparisons were used to determine which treatments differed. The percent contribution of each FA to the separation between diets and among treatments was assessed using similarity percentage (SIMPER) analysis (Clarke, 1993). Analysis of variance (ANOVA) was used to compare FA groups of saturated FAs, MUFA, and PUFA among diets and fish treatments. Post-hoc comparisons of treatment groups were done using Student-Newman–Keuls test (SNK) tests.

3. Results

3.1. Diet FA composition

Diets fed to black bream differed in their FA composition (PERMANOVA, μg FA mg^{-1} dry weight: $P = 0.002$). Differences among diets were driven largely by several PUFAs, which contributed to 43% of the difference between diets, and saturated FAs and MUFAs contributed to 24% difference each (Fig. 1). Several individual FAs contributed substantially to the differences between diets, with these being palmitic acid (16:0), monounsaturated palmitoleic acid (16:1n7c), timnodonic acid (20:5n3), and other PUFA, such as 22:6n3, all of which contributed >10% to differences among diets (Fig. 1). In total, 15 individual FAs contributed to the differences among the two diets signatures.

3.2. Fish FA percentage data

Fish FA signatures, expressed as a percentage, differed according to the diet they were fed (Fig. 2; Table 2), and were unaffected by temperature and feeding duration on the diets. Differences in fish FAs between the diet treatments were dominated by PUFA (43% from 13 FA) in terms of the percentage contribution of individual FAs. Several PUFAs contributed >4% to the differences among diets, with these being 18:2n6, decosahexaenoic acid (22:6n3), and timnodonic acid (20:5n3). MUFA and saturated FA also contributed to differences among diet treatments, however, their overall contribution was less than PUFA (19% and 28% for saturated FA and MUFA) with fewer individual FAs in each group (8 saturated FA all contributing <~3%, 12 MUFA all contributing <~4%). Of interest is that the FAs that contributed to differences among