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Stable isotope determination in wild and farmed gilthead sea bream (*Sparus aurata*) tissues from the western Mediterranean

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Abstract

Stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) have been determined in wild and farmed gilthead sea bream (*Sparus aurata*) samples of white and red muscle, liver, gills and gonads. First, $\delta^{13}C$ and $\delta^{15}N$ values were determined in samples with and without lipid removal to check the possible effect of lipid content on the stable isotope values of the different tissues studied. Differences were found for $\delta^{13}C$ in all tissues studied apart from white muscle of wild fish, the tissue with the lowest lipid content. For $\delta^{15}N$ values no differences were found in wild fish tissues. Liver from farmed fish showed lower $\delta^{15}N$ value after lipid removing. Further conclusions were based on results obtained from lipid-free samples. $\delta^{13}C$ of cultured fish tissues showed a mean depletion of $2.9 \pm 0.4‰$ compared to wild specimens, suggesting different sources of carbon in the diet, probably due to the feed used during sea-cage culture. Cultured gilthead sea bream tissues were significantly more enriched in nitrogen than wild specimens by an average of $1.5 \pm 0.2‰$ in white muscle, indicating a slight increase in the trophic level. Determination of stable isotope signatures of gilthead sea bream tissues allows clear discrimination between wild and cultured sea bream, and characterisation of differences in diet and feeding conditions in any tissue studied.
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1. Introduction

Marine aquaculture has seen strong development in the last few decades as a consequence of increased fish consumption by the world population and decreasing wild stocks. Fish culture has also increased in the western Mediterranean, where it is mainly focused on two species: gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). Fish culture operates in parallel to traditional fisheries and nowadays both cultured and wild fish are important components of the Mediterranean diet.

Different aspects of the operation of sea-cage marine farms have caused considerable concern including coastal environmental impact (Iwama, 1991; Wu, 1995; Vizzini and Mazzola, 2004) and escapes of cultured specimens to the environment (Dempson and Power, 2004). Fish from sea-cage and land-based farm productions show significant organoleptic differences in many species (Prescott and Bell, 1992; Webster et al., 1993; Sylvia et al., 1995). Differences between the organoleptic characteristics of wild and farmed gilthead sea bream due to the different volatile aroma compounds and fatty acid profiles of the fish muscle have been reported (Grigorakis et al., 2003). On the other hand, differential bioaccumulation of lipophyllic pollutants, such as organochlorine compounds, has been found between wild and farmed fish (Easton et al., 2002; Antunes and Gil, 2004). It is known that factors such as lipid level in the diet (Hemre and Sandness, 1999) and ration level (Johansson et al., 1995; Hillestad et al., 1998) increase the body lipid composition and can consequently account for the different fat content and other related differences observed between wild and farmed specimens.

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Stable isotope analysis represents a powerful tool to study feeding relationships in aquatic environments, based on the assumption that a predator contains heavier N and C isotopes (^{15}N and ^{13}C, respectively) than what it feeds on. It is also accepted that the source of C is reflected in changes of ^{13}C value (Michener and Schell, 1994; Smit, 2001). Therefore, this technique should be applied to elucidate the different characteristics that arise as a consequence of culture conditions between wild and farmed fish. And in fact, stable isotopes have been used to distinguish between wild and farmed salmon (Dempson and Power, 2004).

The variations among the stable isotope signatures of the different tissues can be attributed to different causes. Tissue lipid content clearly affects its $\delta^{13}C$ isotopic ratio (17), while the effect on $\delta^{15}N$ depends on tissue and lipid content (Lorrain et al., 2002; Soritopoulos et al., 2004). Isotopic enrichment also depends on tissue turnover rates, and can vary among a given type of tissue (Lorrain et al., 2002; Bodin et al., 2006).

We hypothesise that fish produced by marine aquaculture facilities present different feeding relationships to wild population. This can be characterised by the stable isotope technique, which provides information about their trophic position and the origin of the differences found between them.

The last technological advances have given to scientists the possibility of the measurement of natural abundance of stable isotopes and its application in different scientific areas. Particularly, the determination of carbon and nitrogen stable isotope ratios in live organisms provides a powerful tool to the characterisation of web food steps and give us information about sources of natural carbon and nitrogen entering in the ecosystem (see Michener and Schell, 1994; Smit, 2001).

In the present study, the effect of lipid removal on nitrogen and carbon stable isotope ratios in fish tissues is determined to eliminate lipid content as a factor influencing in the results. Following this we determined $\delta^{13}C$ and $\delta^{15}N$ in lipid-free samples of white and red muscle, liver, gills and gonads of wild and farmed specimens of gilthead sea bream to elucidate the different feeding and trophic characteristics between natural and cultured specimens. The data obtained in this study are useful for improving both the knowledge about feeding and about the trophic consequences of fish production in captivity. This information may provide a baseline for future studies and models of feeding relationships within the new food chain that arise from marine aquaculture activities.

2. Material and methods

2.1. Sample collection

Real world samples of wild and cultured gilthead sea bream (*Sparus aurata*) came from populations located off the Castellón coast (Spanish Mediterranean coast) (Fig. 1) either by trawl catch, or from sea-cage farms located in the same area. They were purchased from local commercial markets, dissected, the target tissues separated, and stored at −20 °C until analysis.

As consequence of the possible interaction between farms and environment, as escaped specimens from farms, or wild fish feed residues from farms, additional checking besides commercial identification was carried out. Morphology and lipid tissues content were used to confirm the fish origin.

Samples were collected in October 2005. Composite samples were made up from six individual specimens (female specimens around 500 g) that were pooled to obtain homogeneous composite samples of each tissue studied. These samples were analysed in six tuplicate for each tissue with and without lipid extraction.

2.2. Sample preparation

Lipids were extracted, and determined by gravimetry, from a half of each sample by refluxing ca. 8 g homogenized unfrozen sample in *n*-hexane during 4 h. Then all samples, with and without lipids, were dried at a constant temperature of approximately 60 °C for 48 h. The samples were ground to an ultra-fine powder using mill Super JS (Moulinex, France).

2.3. Isotope analysis

For stable isotope analysis, approximately 1 and 0.1 mg of homogeneous dried material was weighed into 5 × 9 mm tin capsules for the determination of $\delta^{15}N$ and $\delta^{13}C$ respectively. Samples were analysed for stable isotope ratios of carbon and nitrogen using an isotope ratio mass spectrometer Micromass Isoprime with an elemental analyser EuroEA 3000.

Isotope ratios are expressed in conventional δ notation in per thousand (‰) relative to universal standard:

$$\delta X_{\text{sample}} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1\right] \times 1000$$

All results are reported with respect to VPDB (cretaceous Vienna PeeDee Belemnite) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. The analytical precisions were ±0.20 and ±0.25‰ for nitrogen and carbon, estimated from the standards IAEA-N1 and USGS 24 (graphite) respectively, which were analysed together with the samples.

2.4. Statistical analysis

$\delta^{13}C$ and $\delta^{15}N$ values for tissues before and after lipid removal were compared by means of the t-Student test. Data of stable isotopic composition on different tissues after lipid removal were analysed by means of ANOVA I and "*a posteriori*" Scheffe's test ($P < 0.05$). Homoscedasticity of variances was tested by means of Bartlett's test ($P < 0.05$). Precisions between replicates were calculated as standard deviation, if another parameter is not indicated. All the

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