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Preliminary evidences of colour differences in European sea bass reared under organic protocols

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ABSTRACT

Product diversification, among which organic farming, is an important issue in modern aquaculture activities. Discriminating organic *vs.* conventional products is complex, but appearance may help in tracing different batches of produce. To test this fact, sea basses were fed a commercial or an organic diet, and fishes of each different group were photographed before and during the experiment. Body landmarks were digitized on each colour-calibrated (using the TPS-3D algorithm) image; on the basis of landmarks configuration, the RGB matrices were warped using a geometric morphometrics procedure. The calibrated colour matrix of each warped individual (195 × 135,225) was analyzed with a 50–50 MANOVA, followed by a partial least squares discriminant analysis. Finally, a cluster analysis on the diet/time groups was performed. Growth and changes in condition factor over time are not dependent on the rearing method. Colour (as represented by the pixel vector) does depend on time and on rearing method, based on the MANOVA method used. Standard length and condition factor were not good predictors of colour. The partial least square discriminant analysis was highly effective in detecting colour differences on the basis of the fish diet. The 9-group dendrogram showed that the wild sample and the organic fish cluster together. The head, darker in fishes raised conventionally, is the part showing the greatest difference; the longer the life spent under the 2 regimens, the stronger the differences. In conclusion, these preliminary results demonstrate that a colorimetric analysis is able to distinguish 2 batches of fishes fed different diets in different environmental conditions and – in the present instance – to certify the organically grown sea basses.

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1. Introduction

In many instances, particularly in Europe and North America, current aquaculture productions are near market saturation; nevertheless, revenues can still be increased through diversification, both in terms of new products and of value-adding preparations. In the last years, this diversification goal has been pursued at the beginning of the pipeline – *e.g.*, raising alternative, niche and "minor" species (Engelsen et al., 2004) – or at the consumer end, offering fresh and frozen fillets, fish patties, ready-to-cook preparations and so on (Bianchini et al., 2010). In fact, product diversification could be reached during the raising phase too, employing organic farming protocols (Subasinghe, 2003); while organic fish farming is less than 1% of the aquaculture total output, it is nevertheless worth more than 50 million euros per year (IFOAM, 2010).

Organic fish farming is based on a few major strongholds, of which some are already operative, and others will enter in force only in the next future (European Community, 2009):

- the fish feed must be made with organic components: this requirement is of difficult application for the fish meal and oil

Abbreviations: ANOVA, analysis of the variance; C, conventional rearing protocol; CF, condition factor; exVarSS, (sum of SS for each response)/(sum of total SS for each response); exVarBuf, variance explained by (nPC + nBuf) components; LV, latent vectors; MANOVA, multivariate analysis of the variance; nBuf, number of principal components used as buffer components; O, organic rearing protocol; PC, principal components; PCA, principal component analysis; PLS, partial least squares; PLSDA, partial least squares discriminant analysis; RMSE, root mean square error; RPD, ratio of percentage deviation; SEP, standard error of prevision; ROI, region of interest; SPXY, set partitioning based on joint *x*–*y* distances; SL, standard length; sRGB, standard Red Green Blue colour space; SS, sum of squares; StD, standard deviation; TPS-3D, 3-dimensional thin plate spline algorithm.

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components, while it is easily accomplished for the vegetable foodstuffs
using produce (or leftovers) from organic agriculture,
being meals from terrestrial animals avoided as a rule;
- the farming environment must be "fish-friendly", with plenty of current water and a lower stocking density than usual;
- fry and fingerlings must originate from organic broodstock, and all the reproduction steps (maturation, fertilization, hatching, weaning, *etc.*) shall conform to the "natural" way;
- use of allopathic substances must be reduced to unavoidable veterinary treatments;
- practices must be of minimum impact to the environment before, during and after farming.

Extensive and semi-intensive fish farming are not always synonymous with organic aquaculture: in fact, organic produce must be certifiable, and certified, as such, *i.e.* the certification is an integral part of the organic production chain. Fish raised in "natural" conditions, *e.g.*, those from the Italian "valli" or from managed lagoons (Costa et al., 1990) are not, *ipso facto*, "organic".

Certification and labelling (IFOAM, 2010) are essential parts of the organic pipeline, as well as the controls after the product has been fished; in fact, it would be almost impossible otherwise to discriminate a fish grown organically from another raised with conventional practices, without recurring to long and expensive analyses.

Appearance is used throughout the production, storage, marketing and utilization chain for sorting by size or possible malformations (Bianchini et al., 1994; Costa et al., 2013a), but also as the immediate tool for judging the overall quality of the product, and may even be used in tracing the fish origin (Costa et al., 2011). Fish appearance can be due to many different factors such as optical properties, physical form and health status, chemical composition and microbial load, method of slaughtering and preservation and the environmental conditions in which it has lived (Costa et al., 2013a).

Since the consumer is the ultimate judge of quality, most instrumental methods must be correlated with sensorial measures related to the sight, the touch or the odour perception (Menesatti et al., 2010; Quevedo et al., 2010). For whole fish the EU quality grading scheme (Howgate et al., 1992) is used as required by EU regulation (European Community, 1996) but some initiatives have been taken to implement a new sensory method named Quality Index Method (QIM) to standardize sensory assessment for each species (Martinsdottir et al., 2004; Olafsdottir et al., 2004). Sensory attributes influencing the freshness and quality of fish related to appearance, texture, smell, colour, defects and handling were all considered very important (Quevedo et al., 2010).

Generally, species-specific colour is a critical sensory characteristic of fish quality as it is used by consumers as an indicator of the perceived quality and freshness. All sets of colour values show a fairly good linear relationship with both the QIM values and the values for appearance of skin (Olafsdottir et al., 2004). The functioning of modern colorimeters is comparable to the principle of colour perception used by the human eye (Li-Tsang et al., 2003).

In this scenario, the scope of the present work is to test if the colour appearance can be employed to distinguish European sea basses (*Dicentrarchus labrax L.*) reared using the conventional method (C) from the fish grown under an organic protocol (O). This testing has been conducted coupling colour calibration and multivariate modelling techniques, with the aim of implementing a simple, rapid, cheap, non-invasive method for quality certification and for fish traceability along the supply pipeline.

2. Materials and methods

2.1. Experimental setup

The experiment was carried out in an experimental facility on the Lake of Sabaudia (LT, Italy), a coastal lagoon 100 km SE of Rome; two indoor square tanks (2 m × 2 m × 1 m, but in fact holding just about 2 m^3 of water) were filled with brackish water coming from the connecting channel; the circulation was thru an open system (20–60 l/min, *i.e.*, 15–45 replacements/d) subject to natural external conditions, with ample seasonal and tidal variations in temperature (14.5 °C in November, 29.2 °C in July) and salinity (23.1‰ at low tide, 37.8‰ at high tide).

275 young-of-the-year sea basses, *D. labrax* L. 1758, ranging 140–280 g, were collected from the fishing weir and placed in the tanks at different densities, *i.e.*, 87 animals m^{-3} and 55 animals m^{-3}, respectively for the conventional protocol (C) and the organic protocol (O).

Apart from the stocking densities, fishes in the conventional protocol were fed with a commercial diet (Ytelse M 664, 4.5 mm), while fishes under the organic protocol were supplied with a special feed (EcoLife Pearl 864, 4.5 mm) of the same manufacturer (Biomar sas, Nersac, France). Table 1 reports the basic ingredients, proximate composition and principal energy characteristics of the two feeds.

The experiment started in March 2011 (T_0), when unweaned sea basses were photographed and measured, and the differential feeding began (conventional *vs.* organic, C *vs.* O). Images of animals were acquired 4 times, approximately 6 weeks intervals, during the rearing period (from T_1 to T_4). Fishes were returned back into the tanks after image acquisition. Table 2 reports the number of images acquired and validated for each rearing treatment (C *vs.* O) and time (from T_0 to T_4), together with average standard length (SL, cm), Fulton's condition factor (CF = 100 W SL^{-3}) and their respective standard deviation (StD); these data were used as parameters in the statistical analyses.

Matter-of-factly, the performances in weight increase of the sea basses raised with the conventional and organic protocols are very similar, as shown in Fig. 1.

2.2. Data collection

In order to measure the fish colour pattern, a total of 195 images (Table 2) of individual fish were acquired; the animals were

Table 1
Ingredients, proximate composition and energy characteristics of conventional (Ytelse M 664) and organic (EcoLife Pearl 864) sea bass feeds (from the manufacturer's notice).

	Conv.	Organic		Conv.	Organic
Proteins (%)	44.0	46.0	Total energy (MJ)	22.5	20.0
Lipids (%)	20.0	15.0	Digestible energy (MJ)	19.7	17.0
Carbohydrates (%)	22.0	17.0	Energy from proteins (%)	51.0	58.0
Ash (%)	6.5	41.0	Energy from fats (%)	41.0	32.0
Water (%)	7.5	11.6	Energy from starches (%)	8.0	10.0
Of which cellulose (%)	2.7	1.4	Proteins/energy (g MJ^{-1})	21.7	24.3

Ytelse: corn gluten, peas, soya cake, fish meal, rapeseed cake, fish oil, rapeseed oil, peanut cake, minerals, vitamins.
EcoLife: fish meal, organic peas, organic soya cake, fish oil, minerals, vitamins.

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