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Farming *Sparus aurata* (Teleostei: Sparidae) in marsh ponds: trophic characterization and trace metal accumulation

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ABSTRACT

Considering the overexploitation of fishing on most of the world coasts, the ingestion of fish and shellfish will depend mostly on aquaculture production. Since intensive mariculture usually involves environmental impact, developing sustainable cultures is a priority. In this sense, salt marshes can provide ecosystem services and incorporate both conservation and extensive aquaculture activities. In the present study we compared gilthead seabream *Sparus aurata* Linnaeus, 1758 cultured in extensive and semi-intensive marsh ponds with wild conspecifics from surrounding coastal areas, using trophic characterization (diet analysis and stable isotopes) and trace metal accumulation. Stomach content analysis revealed different feeding habits among gilthead seabream from different origin. Although wild specimens had the most diverse diet, results of stable isotopes showed that extensive diet had the wider isotopic niche and revealed the highest similarities between wild and extensively cultured gilthead seabream. A similar trace element signature was also measured in wild and extensive culture, whereas the semi-intensive culture showed different concentrations for several elements. Cr, Fe and Mn showed the highest concentrations in semi-intensive cultured fish, while As and Zn showed the lowest values in this group. In any case, average values measured in both extensive and semi-intensive culture were, in general terms, below the hazardous limits provided by the standards recommended for trace metals by national and international regulations. Therefore, marsh ponds provide a suitable environment where the cultured fish, especially extensive, should be promoted.

1. Introduction

Aquaculture is one of the most important growing food sectors on earth (Ahmed et al., 2019; Tacon, 2020) and production is increasing at an average annual rate higher than 6% per year (FAO Fisheries Department, 2019). In parallel, the concern for the environmental implications of intensive mariculture has also increased, and environmental impact is often considered when aquaculture activities are established (Tovar et al., 2000; Ahmed et al., 2019). In this sense, developing integrated and sustainable aquaculture in salt marshes can provide multiple ecosystem services and incorporate both conservation and extensive aquaculture activities (Walton et al., 2015). Salt marshes are important wetland areas in temperate zones (Cañavate et al., 2015), which serve as critical habitats for species harvested by fisheries and as nurseries for nekton (Minello et al., 2003; Baker et al., 2020). Historical marsh losses, associated with increasing pressures from coastal development and climate change, place these ecosystems under growing threat (Gedan et al., 2009). However, although many salt ponds around the world have begun to be abandoned because of low profitability (Athearn et al., 2012), the management of these environments for extensive aquaculture is a traditional activity in the Mediterranean (e.g. Labourg, 1976; Ardizzone et al., 1988; Arias and Drake, 1993) with increasing interest during the last years in the context of the sustainable aquaculture. In this sense, similar aquaculture activities on this type of

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ecosystems have been developed in many countries worldwide, including France, Spain, Portugal, Italy, Sri Lanka and United States among others (Amanieu, 1973; Clement, 1986; Gamito, 1989; Athearn et al., 2012 and references therein).

In southern Spain these promising ecosystems are locally named 'esteros'. A typical salt-pond property comprises a first reservoir pond that takes the water directly from the sea channel throughout a pond-monk, followed by a labyrinth-shaped evaporation ponds system and finally by the crystallisation ponds. The water column in the ponds system is progressively lower from 1 to 1.5 m in the reservoir to just few centimeters in the crystallisation ponds. The reservoir ponds can be defined as seawater reservoirs built by enclosing a piece of salt marsh to guarantee a constant supply of water from the saltworks (Izquierdo et al., 1997). These reservoir ponds accounts for almost a third of the total surface of the salt marsh, and they are the place traditionally used for fish farming (Yúfera and Arias, 2010). The diverse macroinvertebrate community, including small molluscs, crustaceans (mainly peracarids), polychaetes and chironomids among others, provides the main source of food for non-intensively reared fish (Arias and Drake, 1994). In fact, 'esteros' have been proposed as an interesting example of Integrate Multitrophic Aquaculture (IMTA) systems combining the extensive culture of fish and crustacean amphipods associated to the marsh ponds (see Jiménez-Prada et al., 2018, 2020 for details).

Two types of culture are being developed in marsh ponds: (i) Extensive ponds, when fish species exclusively feed on invertebrates or small fishes/larvae that naturally inhabit the ponds without supply of commercial aquafeeds, and they are usually stocked at very low densities (<2 kg/m^3); and (ii) Semi-intensive ponds, when fish species feed mainly on supplied commercial aquafeeds but can also feed on the natural preys inhabiting the ponds, and they are stocked at low densities (2–5 kg/m^3). Semi-intensive-ponds are less ecologically sustainable than extensive ponds, since require the use of commercial aquafeeds and it involves an extra contribution of nutrients and organic enrichment of the system. Furthermore, extensive ponds promote the welfare of cultured fish since they have more space and are stimulated by foraging and feeding on natural prey.

The farming procedure in these marsh ponds consists on opening the pond-monks for several months during winter and spring (Yúfera and Arias, 2010), allowing the water flowing free through the reservoir and salt ponds by the action of tides (0.5 up to 3.5 m level difference in this geographical area). After this period of natural recruitment, the pond-monks are almost permanently closed allowing only a partial and episodic water renovation through the monks equipped with nets to prevent the fish escaping. Fish larvae trapped in the ponds grow during several months/years and fed mainly on either natural benthic communities and small fish (extensive culture) or artificially on commercial aquafeeds supplied to the 'estero' (semi-intensive culture). In specific occasions, ponds can be restocked with juvenile fish from nearby hatcheries in order to reach the desirable population density.

Fish cultured in marsh ponds are usually produced in a polyculture system (e.g. mugilids, seabream, seabass and sole), specially under extensive conditions, but monoculture of gilthead seabream (*Sparus aurata*) is being increasingly implemented in marsh ponds (Drake and Arias, 1997; Tovar et al., 2000; Ferrón et al., 2007). Gilthead seabream has great interest for aquaculture mainly due to the high yields and considerable commercial value. It has been successfully cultured in many parts of the world, mostly throughout the Mediterranean basin (mainly in Greece, Turkey, Italy and Spain) (Grigorakis et al., 2002; Arechavala-Lopez et al., 2020). Several techniques have been developed to discriminate the wild or farmed origin of fish by quantifying differences between genetics, chemical characteristics, fatty acid compositions, trace elements, pollutants, stable isotopes, morphology and organoleptic characteristics (Arechavala-Lopez et al., 2013). In fact, extensive research has been conducted to compare wild and farmed gilthead seabream (Arechavala-Lopez et al., 2013 and references therein), mainly regarding the fatty acid profile and external appearance and morphology (Alasalvar et al., 2002; Grigorakis et al., 2002, 2003; Mnari et al., 2007; Yildiz et al., 2008; Del Coco et al., 2009; Lenas et al., 2011; Rogdakis et al., 2011: Šimat et al., 2012; Bodur, 2018). However, most of the studies have focused on wild *vs* intensive farmed fish cultured in open sea aquaculture facilities (floating sea-cages), and there is a remarkable lack of knowledge regarding semi-intensive and, especially, extensive cultures (Flos et al., 2002; Orban et al., 2003; Del Coco et al., 2009; Cretì et al., 2010; Rogdakis et al., 2011). Although there is some evidence that gilthead seabream from different rearing systems has different characteristics, very little information exists (Matos et al., 2017). This contrasts with the increasing interest of developing extensive, responsible, and sustainable gilthead seabream aquaculture in marsh ponds. This lack of information prevents adequate management strategies to promote economic and environmental sustainability programs, development of promotional and marketing campaigns based on scientific evidence, and establishment of standardized quality seals for fish cultured under these ecological requirements. Therefore, this study aims to compare wild-caught (W) gilthead seabream with conspecifics from extensive (E) and semi-intensive (S) ponds, through an integrate approach using diet characterization, stable isotopes, and trace metal accumulation. Additionally, trace metal concentrations measured in the present study are compared with standard health limits for human consumption recommended by different national and international regulations, including European Commission (EC), World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) among others.

2. Material and methods

2.1. Study area and sampling

The study was conducted in the Bay of Cádiz (Southern Spain, Fig. 1), where the fishery has an important economic and employment role (Miró et al., 2020). Indeed, the bay is surrounded by a vast area of marshes traditionally exploited for natural resources, salt and seafood. Transformation of these marshes for salt extraction, already documented several centuries ago, was intensified during 18th and 19th centuries (Yúfera and Arias, 2010). As a result of these activities there is presently a complex system of sea channels and creeks that supply seawater to the saltmarsh fish-pond systems situated along their courses. Its biological richness explains its use as a natural nursery area for fish coastal communities (Yúfera and Arias, 2010). Most of these saltmarsh ponds remain continuously inundated during a major part of the year and constitute a semi-natural lagoon ecosystem exploited for extensive and semi-intensive fish culture (Arias and Drake, 1994). Demographic concentration and the development of economic activities compete for very scarce space due to the geographical characteristics of the coast and also to the presence of a protected area (Bay of Cádiz Natural Park) which has valuable ecosystems for the services they provide to neighboring cities (De Andrés et al., 2018; Vázquez-Pinillos and Marchena-Gómez, 2021). This bay is a natural nursery habitat for gilthead seabream and provides favorable and stable habitat for juveniles (Sánchez-Lamadrid, 2004 and references therein), being an important area for local professional fishing of this species (Muñoz-Pérez and Sánchez-Lamadrid, 1994). In this context, the sustainable use of 'esteros' for aquaculture of this species is especially remarkable and the study area is an optimal scenario to compare wild and farmed specimens.

For the present study, two marsh ponds ('esteros') were selected according to the type of *S. aurata* culture. On the one hand, an extensive pond located on the bank of the San Pedro River between Puerto de Santa María and Puerto Real localities was chosen (Fig. 1A). San Pedro River area is a shallow tidal creek located within the salt marsh area of the Bay of Cádiz (SW Spain) (36°23′–37′N, 6°8′–15′W). It is characterized by semi-diurnal mesotides (average tidal range 0.98–3.20 m) and the tidal current flows from the bay along the creek where the freshwater inflow is not significant, except during heavy rains (Ferrón et al.,

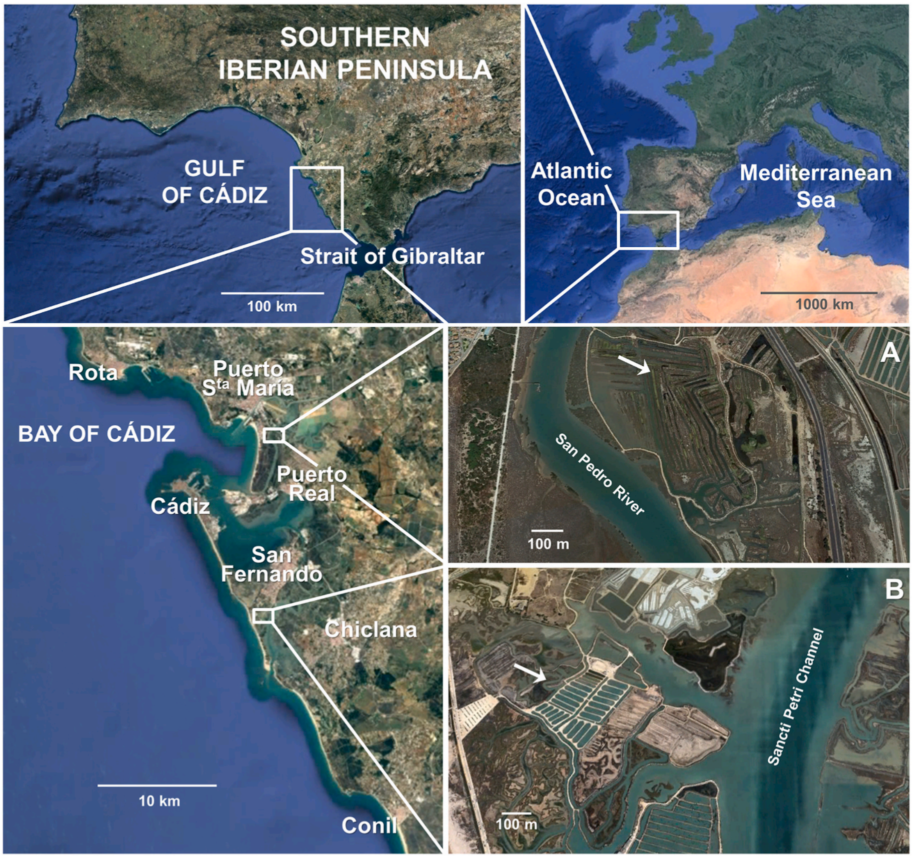

Fig. 1. Study area showing the location of the extensive (A) and semi-intensive (B) 'esteros'. Wild specimens were collected along the coast between Rota and Conil localities.

2007). The creek is 12 km long, with a width ranging from 15 to 30 m and a maximum depth of 5–6 m, and the water column is well mixed with no significant salinity differences between the surface and the bottom (González-Gordillo et al., 2003). The system receives the inputs of organic matter and nutrients coming from the wastewater discharges of several fish farms located in the area (Tovar et al., 2000; Ferrón et al., 2007, 2009). Fish from this extensive pond fed only on invertebrates naturally inhabiting the ponds and no commercial aquafeed was supplied. On the other hand, the selected semi-intensive pond, belonging to CULMINSA company, is flooded by waters coming from Sancti Petri Channel, in San Fernando locality (Fig. 1B). The Sancti Petri tidal channel is an inflow-outflow channel that extends from the inner part of Bay of Cádiz to the outlet. It is 17 km long and connects to several secondary channels that, in turn, supply a vast tidal flat area (Vidal and Tejedor, 2005). The depth ranges from approximately 9 m in the central area to 3 m in the margins; the channel bed is covered with cohesive sediment, mud and clay (Gutiérrez et al., 1996). Fish from the semi-intensive 'estero' were fed on artificial fish food (commercial aquafeed DIBAQ®) along with natural diets that fish could find in the 'estero'. Due to confidential policy, there was no more information available about the commercial feed and the current ratio with natural diets.

A total of thirty gilthead seabream specimens were collected from extensive ponds (E) and another thirty fish from semi-intensive ponds (S). Similarly, thirty wild gilthead seabream (W) were caught by local fishermen using gill net fishing from the coast of the outer Bay of Cádiz and nearby areas (from Rota to Conil, Fig. 1). Fishing of wild specimens was carried out from 2 to 11 September 2015 at 10–20 m depth. The anonymity of the exact locations where the fish was collected is preserved, according to the wishes of the local fishermen. Fish collected from the extensive 'estero' were captured 9 and 10 November 2015 from ponds at 1.5–2 m depth. Specimens from the semi-intensive 'estero' were collected on 16 December 2015, at 1–3 m depth. Immediately after purchasing, all dead fish specimens were placed into separate polyethylene bags, individually labeled, and brought to the laboratory in icebox. Since live animal samples were not included in the research, the study did not require ethical approval.

Once the specimens arrived at the laboratory, body length (BL, in cm) and body wet weight (BW, in g) of each fish were measured and recorded. Then, fish were washed with sterile distilled water and immediately dissected with aseptic plastic forceps and knife. Stomachs (from esophagus to pyloric sphincter) were fixed and preserved in ethanol 70% to further study (Arechavala-Lopez et al., 2012). Muscle samples were divided into sections and kept frozen at −80 °C. Previously to analyses of stable isotopes and trace metals, samples were freeze-dried for 48 h into a ilShin Biobase Europe lyophilicer model FD8512 to constant weight.

2.2. Gut contents

Preserved stomachs of the 90 specimens (30 of each fish group W, E and S) were opened, and the contents examined and clustered according to major taxonomic groups. The number of empty stomachs was recorded, and prey identification was carried out to the lowest possible taxonomy level (Chaouch et al., 2013). Dietary items were weighted after removal of surface water by blotting paper (Arechavala-Lopez et al., 2012). The wet weight of each prey was used as quantification measure since in many cases only pieces of prey could be detected, and it was not possible to provide an accurate counting of number of specimens of each one. The following indices were used to characterize the diet: (i) Frequency of Occurrence (%O = 100 × [number of stomachs containing prey i/total number of stomachs containing prey]); (ii) Percentage

Weight (%w = 100 × [weight of prey i/total weight of all prey]); (iii) Vacuity Index (VI = 100 × [number of empty stomachs/total number of stomachs analyzed]) (see Arechavala-Lopez et al., 2012, and references therein).

2.3. Stable isotopes

Muscle samples of 60 specimens (20 of each fish group selected randomly from the 30 available) were used for stable isotopes. Freeze-dried samples were milled to fine powder using a ball mill Retsch MM400. Subsamples of powdered material was weighted to the nearest 0.300 mg with an error of ±0.002 mg and placed into tin capsules for $\delta^{13}C$ and $\delta^{15}N$ determinations. All samples were combusted at 1020 °C using a continuous-flow isotope-ratio mass spectrometry system by means of Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope ratio mass spectrometer via a CONFLO IV interface (Thermo Fisher Scientific, Bremen, Germany).

2.4. Trace metals

Muscle samples of 45 specimens (15 of each fish group selected randomly from the 30 available) were used for analysis of trace metals (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn). Freeze-dried samples (approximately 500 mg) were mineralized in Teflon digestion vessels, in a closed microwave digestion using 0.5 ml of nitric acid HNO_3 and 1.5 ml of hydrogen peroxide H_2O_2 as reagents (Suprapur® grade, Merck, Darmstadt, Germany). Quantification of elements in the extracts was achieved using a VARIAN ICP 720-ES (simultaneous ICP-OES with axially viewed plasma), equipped with ultrasonic nebulizer CETAC U5000AT+. Standard solutions for the devices' calibration were used. For preparation of standards, <18 MΩ/cm ultrapure water supplied from a Milli-Q Millipore system were used (Bedford, MA, USA) and TracepureTM HNO_3 from Merck (Darmstadt, Germany). Calibration and Quality Control (QC) solutions were prepared from an ICP multi-element standard solution IV Certipur obtained from Merck and Spectrascan certified reference solution from LGC Standards GmbH (Wesel, Germany). To prevent contamination of the samples with any traces of metal, all material used for sample storing and treatments and all labware equipment was soaked in 2% v/v HNO_3 solution followed by two washes with Milli-Q water. The calibration blank was prepared with 2% v/v HNO_3. Analytical blanks and standard reference materials were run in the same way as samples. The accuracy of the analytical methods was assessed through reference sample (TORT-2: Lobster Hepatopancreas Reference Material for Trace Metals). The differences in concentrations between analyzed and certified values were <10%.

2.5. Statistical analyses

To compare size and weight among the specimens of the three groups, one-way ANOVA was conducted with the factor 'Type', fixed with three levels (i.e. fish groups: W, E and S). Trace metals were also compared using one-way ANOVA. Prior to ANOVAs, the homogeneity of variances was tested with Cochran's C-test. Where variances remained heterogeneous even after data transformation, untransformed data were still analyzed, as ANOVA is a robust statistical test and is relatively unaffected by the heterogeneity of variances, particularly in balanced experiments (Underwood, 1997). Where ANOVA indicated a significant difference for a given factor, the source of difference was identified using Student-Newman-Keul (SNK) tests. MDS were conducted to show the relationship among types (W, E and S) according to the diet items (wet weight abundance matrix), based on the Bray Curtis similarity. Principal Component Analysis (PCA) was carried out to show the relationship among types (W, E and S) according to trace metals. Data of trace metals were transformed with log (x+1).

Differences among types were also tested using a permutational multivariate analysis of variance (PERMANOVA). Analysis was based on Bray Curtis similarity matrix (diet) and Euclidean distance one (stable isotopes, and trace metals). Significant P values were obtained by computing 9999 permutations under a model of unrestricted permutation of raw data, which is recommended when there is only a single factor (Anderson, 2005). Pairwise comparisons were then used.

Univariate analyses were conducted with GMAV5 (Underwood et al., 2002) and multivariate analyses were carried out using the PRIMER v.6 plus PERMANOVA package (Clarke and Gorley, 2006).

To assess differences in the trophic niche of the three types, analysis of community niche space was performed calculating the standard ellipse area (SEAc) (c means that SEA was corrected for small sample size) and the Bayesian standard ellipse area (SEAb). SEAb were derived from 100,000 posterior iterations and was reported as the mode with 95% credible interval (Jackson et al., 2011). Metrics proposed by Layman et al. (2007a) to describe trophic parameters of populations were also calculated. These metrics include: $\delta^{13}C$ range (CR), $\delta^{15}N$ range (NR), mean distance to centroid (CD), mean nearest neighbour distance (M-NND) and their SD (SD-NND). CR is indicative of niche diversification at the base of food webs. NR is a representation of vertical structure in a food web, larger ranges suggest more trophic levels and degree of trophic diversity. CD provides a measure of the average degree of trophic diversity within a food web. M-NND represents trophic redundancy. Finally, SD-NND is a measure of the evenness of food web, high values suggest more diversification of trophic niches (see Layman et al., 2007a for more details). All measures were calculated using the SIBER package in R.

3. Results

Length and weight of gilthead seabream specimens significantly differed among groups (Table 1). Semi-intensive (S) fish showed the highest body length (S: BL range = 22–25 cm) and body weight (S: BW range = 286–394 g) and fish from extensive culture presented the smallest size in length (E: BL range = 15.5–20.5 cm) and weight (E: BW range = 105–226 g). Wild-caught specimens had intermediate size values (W: BL range = 19.3–23.0 cm, BW range = 193–343 g).

3.1. Gut contents

Regarding the diet composition, the number of empty stomachs (and vacuity index, V.I.) was slightly higher in wild fish than extensive and semi-intensive (Table 2). Stomach content analysis allowed the identification of sixteen different food items, belonging to four major groups (Crustacea, Mollusca, Echinodermata and Osteichthyes) (Table 2). Wild specimens showed the most diverse diet, including a variety of decapods (mainly the ermit crab *Diogenes* sp., and the brachyoures *Inachus* sp. and Portunoidea) and molluscs (mainly the bivalve *Corbula gibba* (Olivi, 1792)), along with the echinoid *Echinocardium cordatum* (Pennant, 1777). The most common prey in gilthead seabream from extensive culture (E) were the crustaceans, with a remarkable contribution of the shrimps *Penaeus kerathurus* (Forskål, 1775) and *Palaemon* sp. Small fish

Table 1

Characteristics of *Sparus aurata* specimens used in this study.***, $p < 0.001$. n.s., not significant; se: standard error of the mean value; n: number of specimens; SNK: Student Newman Keuls.

	n	Body Length (cm)		Body Weight (g)	
		Range	Mean ± se	Range	Mean ± se
Wild (W)	30	19.3–23.0	21.4 ± 0.2	193–343	263 ± 7
Extensive (E)	30	15.5–20.5	17.9 ± 0.2	105–226	150 ± 7
Semi-intensive (S)	30	22.0–25.0	23.4 ± 0.1	286–394	337 ± 6
		one-way ANOVA		one-way ANOVA	
Cochran test (C)		C = 0.57 ($p < 0.01$)		C = 0.40 (n.s.)	
F-statistic		199.09***		204.33***	
SNK		W≠E≠S		W≠E≠S	

Table 2

Diet composition of *Sparus aurata* in the three different groups. %O: frequency of occurrence; %w: percentage of wet weight; -: absence of the food item. Number of empty stomachs and vacuity index values are also included.

Gut contents	Wild		Extensive		Semi-intensive	
	%O	%w	%O	%w	%O	%w
Phyllum Arthropoda						
Subphyllm Crustacea						
Class Malacostraca						
Subclass Copepoda	–	–	5.2	0.1	–	–
Subclass Eumalacostraca						
Order Mysida						
Mysidae sp1	–	–	–	–	6.2	0.3
Mysidae sp2	7.7	0.1	–	–	–	–
Order Decapoda						
Diogenes sp	46.1	5.9	–	–	–	–
Inachus sp	23.1	0.6	–	–	–	–
Palaemon sp.	–	–	5.2	4.7	–	–
Penaeus kerathurus	–	–	42.1	69.7	–	–
Grapsoidea unidentified	7.7	0.1	–	–	–	–
Portunoidea unidentified	7.7	12.9	–	–	–	–
Phyllum Mollusca						
Class Bivalvia						
Abra sp	7.7	4.4	–	–	–	–
Corbula gibba	23.1	25.0	–	–	–	–
Unidentified bivalve	7.7	4.9	–	–	–	–
Class Gastropoda						
Ringicula sp.	7.7	3.3	–	–	–	–
Phyllum Echinodermata						
Class Echinoidea						
Echinocardium cordatum	15.4	3.1	–	–	–	–
Phyllum Cordata						
Fundulus sp	–	–	36.8	20.7	–	–
Fish remains (mainly scales)	–	–	26.3	3.5	81.2	98.4
Unidentified items	61.5	39.7	5.2	1.3	6.2	1.3
Number of stomachs examined	30		30		30	
Number of empty stomachs	18		17		14	
Vacuity index (%)	60.0		56.7		46.7	

belonging to the genus *Fundulus* were also found in stomachs of E-cultured gilthead seabream. Only some mysids and fish remains were found in stomachs of semi-intensive cultured gilthead seabream (S). Pellets from commercial aquafeed could not be detected. Diet composition significant differed among types, according to PERMANOVA results (Table 3). Indeed, MDS plot showed, in general terms, a clear separation among fish groups, with extensive systems being closer to wild-caught than to semi-intensive ones (Fig. 2).

3.2. Stable isotopes

Table 3

Summary of the one-way PERMANOVA results based on diet (% weight of gut items), stable isotopes, and trace metals for different origin of *Sparus aurata* (W: wild; E: extensive; S: semi-intensive). Asterisks indicates significant differences, ***$P < 0.001$. MS = Mean Square.

Source of variation	df	MS	Pseudo-F	*P*	Unique permutations
Diet					
Type (W, E, S)	2	8.037	7.065	0.0003***	9942
Residual	45	1.137			
Total	47				
Pair-wise tests	W≠E≠S				
Stable isotopes					
Type (W, E, S)	2	364.06	233.77	0.0001***	9948
Residual	56	1.5574			
Total	58				
Pair-wise tests	W≠E≠S				
Trace elements					
Type (W, E, S)	2	17.048	14.108	0.0001***	9938
Residual	42	1.2084			
Total	44				
Pair-wise tests	(W = E)≠S				

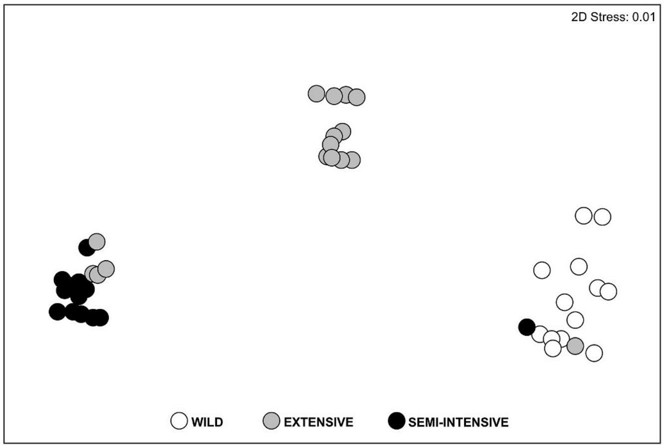

Fig. 2. Non-parametric multidimensional scaling (MDS) plot showing the relationship among seabream groups (wild, extensive, semi-intensive) regarding the stomach content analysis. Wet weight data of diet items were used.

Regarding stable isotopes, a more depleted signal of carbon isotope was obtained for individuals of the semi-intensive culture (S) followed by individuals of the wild (W) and the extensive (E) ones (S: 21.4 ± 0.45, W: 16.32 ± 1.08, E: 14.74 ± 1.14). On the other hand, wild-caught fish (W) showed the highest signal of nitrogen isotope followed by the extensive (E) and semi-intensive (S) cultured fish (W: 14.31 ± 0.80, E: 10.72 ± 1.03, S: 9.31 ± 0.50) (Fig. 3, Table S1). Semi-intensive individuals also showed less variability within the group than the extensive and the wilds on both isotopes (Fig. 3). PERMANOVA results showed significant differences in the bivariate isotopic space for all groups (Table 3). Distribution of the standard ellipse areas (SEAc) of the different gilthead seabream groups within the isotopic space showed differences in their isotopic niche ($\delta^{13}C$-$\delta^{15}N$) (Fig. 3). Isotopic niche varied in position and size between the three groups, being the niche of the wild-caught and the extensive cultured specimens more similar between them and bigger than the semi-intensive cultured fish (Fig. 3). Results of SEAb and SEAc showed that extensive (E) had the wider isotopic niche followed by the wild (W) and the semi-intensive (S). Layman metrics showed widest NR, CR, CD and M-NND in the extensive cultured gilthead seabream, followed closely by the wild-caught fish and clearly differenced from the semi-intensive cultured specimens (Table 4). SD-NND showed greater values in the wild-caught gilthead seabream followed by the extensive group and far away from the semi-intensive cultured fish (Table 4).

3.3. Trace metals

Regarding trace metals analyses in gilthead seabream muscle, PCA analysis showed that wild-caught and extensive cultured fish were more

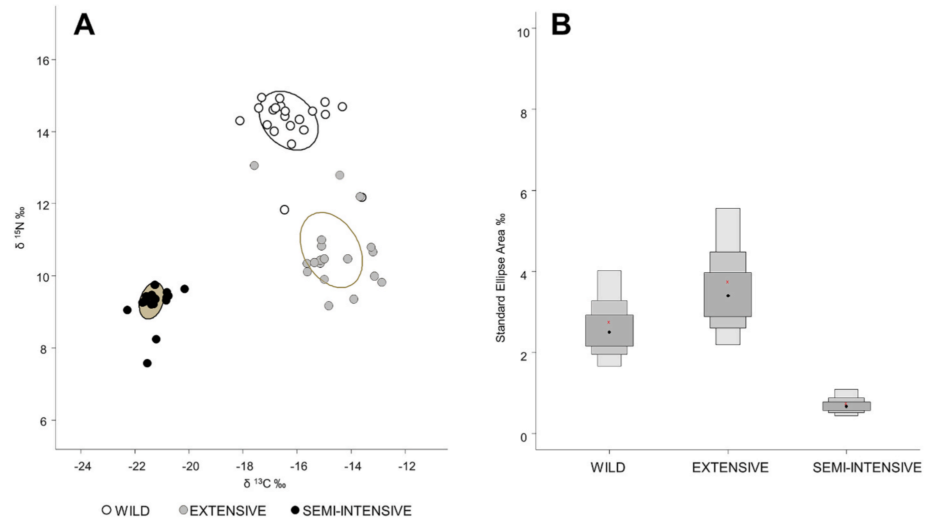

Fig. 3. A) Plot of stable isotopes δ^{13}C (‰) and δ^{15}N (‰) of *Sparus aurata* from muscle tissues and standard ellipse areas corrected for small sample size (SEAc) grouped by diet type. B) Estimated posterior distribution of trophic niche of *Sparus aurata* grouped by type of diet. Black dots are the modes, and boxes indicate the 50%, 75% and 95% credible intervals. The red crosses represent the true population values.

Table 4

Layman's metrics: NR, nitrogen range; CR carbon range; CD, distance to centroid; M-NND, mean of nearest neighbour distance; SD-NND, standard deviation of the nearest neighbour distance (‰), SEAc (standard ellipse area corrected, ‰^{2}) and mode of the SEAb (Bayesian standard ellipse area, ‰^{2}) analyzed in muscle of gilthead seabream with different origins.

	NR	CR	CD	M-NND	SD-NND	SEAc	SEAb
Wild	3.10	4.50	1.07	0.51	0.61	2.75	2.50
Extensive	3.89	4.72	1.31	0.53	0.49	3.73	3.42
Semi-intensive	2.18	2.12	0.52	0.21	0.26	0.73	0.65

similar between them, while semi-intensive cultured individuals presented a more different trace metal composition in muscle (Fig. 4, Table S2). Axis 1 explained 65.4% of the total variance among samples, and correlated with As ($r = -0.898$, $p < 0.001$). This axis clearly separated the wild-caught and extensive cultured specimens from the semi-intensive culture group. Axis 2 explained 12.6% of the total variance among samples, and correlated with Cu ($r = -0.471$, $p < 0.001$), Fe ($r = -0.396$, $p < 0.001$), Ni ($r = -0.466$, $p < 0.001$), Pb ($r = -0.551$, $p < 0.001$) and Zn ($r = -0.284$, $p < 0.05$). The similarities on trace metal composition between wild-caught and extensive cultured fish types, as well as differences between semi-intensive group with the other two groups, were also reflected by the PERMANOVA (Table 3). In this sense, Cr, Fe and Mn showed highest concentrations in semi-intensive cultured fish, while As and Zn showed the lowest values in this group, according to ANOVA analyses (Table 5).

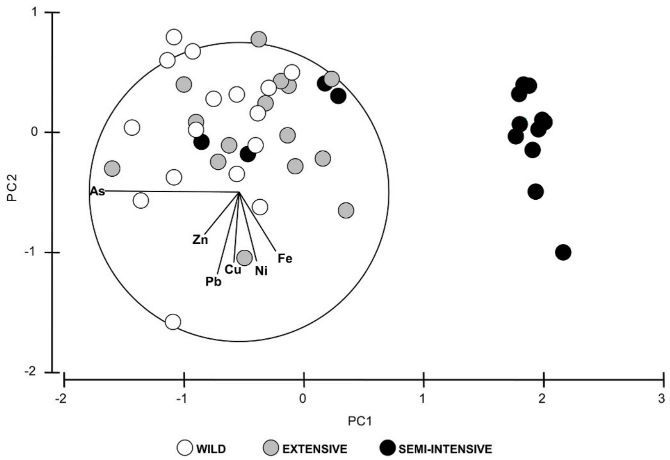

Fig. 4. Principal component analysis (PCA) plot based on the trace metal concentrations analyzed in muscle tissues of seabream from three different groups (wild, extensive and semi-intensive). Only those elements which significantly correlated with the axes PC1 and PC2 were graphically represented.

4. Discussion

Despite of differences in diet and bivariate isotopic space, the present study reveals that gilthead seabream cultured extensively in marsh ponds ('esteros') showed similarities to the wild-caught conspecifics regarding trophic characteristics and trace element concentrations in muscle tissue samples.

4.1. Feeding habits and stable isotopes

Feeding habits assessed through the stomach contents significantly differed among wild-caught, extensive and semi-intensive cultured fish in the present study. Gilthead seabream is an opportunistic feeder and can adapt its diet to the food items available in the habitat (Arechavala-Lopez et al., 2012 and references therein). In agreement with stomach contents found in wild-caught seabream in the present study (Table 2),

Table 5

Trace elements (ppm, dry basis) in the muscle of *Sparus aurata* from different origins. Values are mean ± standard error, n = 15 for each type.*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, n.s.: not significant; SNK: Student Newman Keuls.

	Wild (W)	Extensive (E)	Semi-intensive (S)	ANOVA	
				p	SNK
As	50.11 ± 5.04	34.86 ± 5.02	13.47 ± 4.63	0.000***	W≠E≠S
Cd	0.06 ± 0.01	0.06 ± 0.02	0.02 ± 0.01	0.113 n.s.	W = E = S
Co	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.500 n.s.	W = E = S
Cr	0.28 ± 0.01	0.24 ± 0.02	0.91 ± 0.14	0.000***	(W = E)≠S
Cu	0.44 ± 0.16	0.53 ± 0.18	0.22 ± 0.05	0.315 n.s.	W = E = S
Fe	6.42 ± 0.98	8.51 ± 1.47	11.53 ± 0.95	0.012*	(W = E)≠S
Mn	0.43 ± 0.05	0.41 ± 0.04	1.08 ± 0.22	0.001**	(W = E)≠S
Ni	1.24 ± 0.60	0.64 ± 0.07	1.16 ± 0.20	0.469 n.s.	W = E = S
Pb	1.06 ± 0.21	0.99 ± 0.35	0.33 ± 0.08	0.075 n.s.	W = E = S
Zn	31.86 ± 2.75	30.55 ± 2.15	20.00 ± 1.68	0.001**	(W = E)≠S

previous works on diet characterization of wild seabream revealed that primary prey items are molluscs (bivalves followed by gastropods) and crustacean decapods (Arias, 1980; Rosecchi, 1985, Pita et al., 2002; Tancioni et al., 2003; Chaoui et al., 2005; Arechavala-Lopez et al., 2012). Decapods and small fishes also contributed to the diet of seabream cultured in extensive ponds, but molluscs were not detected in their stomachs. Although the natural preys in semi-intensive culture were restricted to some mysids, a high number of fish scales probably suggests the noticeable contribution of small fish in the diet. In any case, the very low diversity of prey items in this semi-intensive culture clearly indicated that the greatest contribution was derived from the external supply of artificial feed. Unfortunately, food pellets were not observed in the stomachs probably because the feed used (commercial aquafeed DIBAQ®) was breaking up quickly in fine dust and there were no distinguishable remains in the fish stomachs.

As farmed fish diets contain a significant terrestrial carbon input from vegetable meals and oils, stable isotope analysis has been applied to discriminate wild and farm fish (Arechavala-Lopez et al., 2013). Stable isotope analysis integrates the overall diet of individuals over space and time and has been proved to be a powerful tool for the study of population trophic niches (Layman et al., 2007a, 2007b; Jackson et al., 2012). Moreover, this technique is based in the assumption that consumers reflect the isotope composition of assimilated food sources (Garcia et al., 2018). Nitrogen isotope provides information about the trophic position and carbon isotope allows determinate the carbon source and the habitat where the consumers prey. However, stable isotopes also have limitations; it is difficult to distinguish among preys or carbon sources if they have similar isotopic signals. Therefore, the combination of stable isotopes and stomach content analysis can provide complementary information about ingested and assimilated diet and thus, a more effective approach to investigate trophic niche (Giménez et al., 2017; Garcia et al., 2018 and references therein).

The differences in isotopic signals with the different diets obtained during the present study for gilthead seabream are in accordance with previous studies comparing seabream fish from intensive and semi-intensive farms with wild individuals (Morrison et al., 2007; Moreno-Rojas et al., 2010; Serrano et al., 2007; Arechavala-Lopez et al., 2013). The $\delta^{13}C$ showed clear differences between the semi-intensive diet and the other two diet groups. This could be explained by the larger isotopic fractionation of ^{13}C in higher lipid content tissues of the semi-intensive seabream (Serrano et al., 2007; Arechavala-Lopez et al., 2013). Moreover, terrestrial carbon sources in the diets of the semi-intensive seabreams (such as oils of vegetal origin like soybean and sunflower which are often used in aquaculture feed due to economic reasons), could explain the more depleted signals in the semi-intensive diet (Morrison et al., 2007; Moreno-Rojas et al., 2010). Furthermore, differences between extensive cultured individuals and wild ones could be explained because in extensive ponds, seabream could exploit different organic matter sources with a benthic derived organic matter (France, 1995; Duffill Telsnig et al., 2019), and/or with a possible influence of derived organic matter from C4 plants or seagrasses (Feng et al., 2015; Matich et al., 2017; Deng et al., 2018; Wang et al., 2018). Andolina et al. (2020) found that wild subadult seabreams were exploiting resources with a slightly depleted carbon and richer nitrogen signals than the extensive cultured seabream.

Nitrogen signals were significantly higher in wild individuals than in those from extensive and the semi-intensive ponds. The fact that wild seabream presented major trophic level compared to semi-intensive fish is expected and goes in accordance with other studies which have compared wild and farmed seabream and other species (Morrison et al., 2007; Arechavala-Lopez et al., 2013; Gopi et al., 2019). On the contrary, Serrano et al. (2007) found higher nitrogen signal in farmed seabreams and pointed out that this fact were due to the fish origin of the feed in the farmed individuals compared with the natural preys of the wild specimens. In the present study, nitrogen signals of the semi-intensive diet probably indicate a major proportion of vegetal origin in the feed rather than fish, which is in agreement with the carbon isotopes results. Higher nitrogen signal in organisms is also an indicator of anthropogenic nitrogen loads (Morris et al., 2015; Donázar-Aramendía et al., 2019). This finding, together with the depleted signal of carbon in the wild individuals compared to those from extensive ponds could indicate a possible influence of terrigenous enriched inputs of nitrogen in the diet of the wild individuals, explained by the terrestrial loads from the principal estuaries in the Gulf of Cádiz where the wild gilthead seabreams were captured (González-Ortegón et al., 2018). Furthermore, higher nitrogen signal in the wild fish could also be explained by the addition of intermediate consumers or changes in the degree of trophic omnivory in the wild diet compared to the extensive one (Post and Takimoto, 2007).

Gilthead seabream from extensive ponds presented ever higher trophic niche and trophic diversity than wild-caught seabream (although differences were small) probably due to the important contribution of decapods (mainly of the genera *Palaemon* and *Penaeus*) inhabiting the extensive ponds. The natural availability of preys in the marsh ponds (Arias and Drake, 1994) could explain the higher trophic diversity (CD) found in the extensive cultured seabream (Jackson et al., 2012). On the other hand, differences between gilthead seabream from semi-intensive ponds with the other two fish groups were much larger in both trophic niche width and Layman's metrics. Therefore, the artificial feed supply in the semi-intensive gilthead seabreams made their trophic ecology very different to the extensive and wild individuals, which showed similar trophic parameters. Moreover, wild and extensive specimens would have similar diversified feeding behaviour indicated by the similar values of M-NND (Layman et al., 2007a; Abrantes et al., 2014; Andolina et al., 2020). Although gut content significantly differed among fish groups, stomach content analysis can be just a snapshot of the overall diet of species (Matthews and Mazumder, 2004).

4.2. *Trace metals*

A growing concern about the health benefits and risks of food consumption has led to an extensive study of essential and toxic trace element concentrations in foodstuffs (Guérin et al., 2011). The increase in heavy metal concentrations in the aquatic environment due to anthropogenic activities highlights the importance of their measurement because of their toxicity, chronic persistence, bioaccumulation, and biomagnification at different trophic levels (Marengo et al., 2018; Lounas et al., 2021 and references therein).

Marine fish incorporate trace elements directly from the environment or through the diet into their skeletal tissues and organs (Arechavala-Lopez et al., 2013). These elements include essential ones, such as Mn, Fe, Co, Cu, Zn, Ni, Mo, Cr, with important functions (e.g. skeletal structure, maintenance of colloidal system, regulation of acid-base equilibrium, constitution of hormones, enzymes and enzyme activators) (Olmedo et al., 2013). On the other hand, As, Cd, Hg and Pb are non-essential metals, with no known biological role and even toxic in traces for marine organisms and humans (Renieri et al., 2019). Furthermore, the essential metals can also produce toxic effects when the metal intake is excessively elevated (Türkmen et al., 2005). Wild populations of gilthead seabream in the Mediterranean region move among several coastal habitats, and there are no important differences in trace element concentrations among different wild fish populations (Gillanders et al., 2001). In fact, trace metal concentrations are, in general terms, of the same order of magnitude in Mediterranean populations from Turkey, Greece, Italy, France and Spain (see Table 6). We must take into consideration that metal concentrations are expressed in literature either per unit of wet (ww) or dry (dw) tissue weight. Although most of legislation and reference values are provided in ww, most of publications use dw as a measurement unit and a lack of standardized reporting of elemental concentrations obstructs comparability of studies that use different measurement units (Jovičić et al., 2015). Cresson et al. (2017) demonstrated that the theoretical wet:dry ratio of 5

Table 6
Trace metal levels (mg/kg) in muscle tissue of wild (W) and farmed (F) populations of *Sparus aurata* in literature and present study. d: dry weight, w: wet weight, bdl: below detection limit.

Locality	W/F	d/w	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Iskenderun Gulf, Turkey	W	d		4.10–7.60			5.80–10.70	30.70–43.20			14.00–24.60	20.80–32.20	Kargın (1996)
Karataş, Turkey	W	d		0.37		1.24	2.84	19.60			5.54	26.66	Canli and Atli (2003)
Iskenderun Bay, Turkey	W	d		0.09–3.70	0.05–5.61	0.07–3.48	0.08–3.46	4.59–27.35	0.05–4.64	0.25–7.59	0.19–6.23	0.86–11.57	Türkmen et al. (2005)
Iskenderun Bay, Turkey	W	w				0.86–1.08	bdl-0.51	20.65–28.81		0.29–0.87	4.84–6.39	19.31–31.23	Yılmaz (2005)
Homa Lagoon and Mersin Bay, Turkey	W	w		<0.01			0.11–0.38				<0.01–0.01	4.57–5.59	Çelik et al. (2004); Çelik and Oehlenschläger (2005)
Çamlik Lagoon, Turkey	W	d		0.12–0.13				3.25–9.15				33.4–67.75	Dural et al. (2006)
Tuzla Lagoon, Turkey	W	d		0.08–0.12			0.55–0.82	7.16–16.50			0.64–2.44	8.82–76.98	Dural et al. (2007)
Beymelek Lagoon, Turkey	W	w			0.56	bdl	4.31	6.67	0.27	bdl		7.09	Uysal et al. (2008)
Aegen Sea, Turkey	W	d		0.20	2.30	0.50	0.20	3.20	1.20	0.30	0.40	3.10	Yildiz (2008)
Yelkoma Lagoon, Turkey	W	w		0.30	0.28	0.60	0.62	38.9	0.75	0.47	0.45	13.90	Türkmen et al. (2010)
Iskenderun Bay, Turkey	W	w		1.25		0.57	6.24	17.31	2.01		3.83	14.35	Dural et al. (2011)
Akyatan Lagoon, Turkey	W	w		0.07	0.05	0.48	0.62	44.00	0.43	0.50	0.23	7.36	Türkmen et al. (2012)
Trabzon, Turkey	W	w	0.39	3.30				0.58	0.09	bdl	bdl		Aydin and Tokalioğlu (2015)
Hurmabogazi Lagoon, Turkey	W	w		0.13	0.08	0.30	1.33	47.2	1.04	0.20	0.52	11.10	Türkmen et al. (2016)
Heraklion, Greece	W	w		<0.01							0.29		Renieri et al., (2019)
Adriatic Sea, Croatia	W	d	<0.01–0.01	bdl-<0.01	0.02–0.06	0.25–1.60	0.71–1.38	10–16.8	0.31–2.00	bdl-0.1	0.05–0.07	16.0–17.7	Žvad Rožič et al. (2014)
Caorle Lagoon, Venice, Italy	W	w					0.48–3.30	1.30–20.00				5.00–10.50	Carpene et al. (1998)
Ligurian Sea, Italy	W	d	18.40–39.60				1.30–2.20	8.80–19.00	0.30–0.60			15.00–22.00	Minganti et al. (2010)
French coast, France	W	w			<0.01	0.14	0.24	3.16	0.04	0.05	0.01	3.25	Guérin et al. (2011)
Corsica, France	W	w	5.49	<0.01		0.02	0.20	2.17	0.14		<0.01	3.55	Marengo et al. (2018)
Northwest Mediterranean, France	W	w	4.94–23.71	bdl	<0.01	<0.01–0.02			0.04–0.09	<0.01	<0.01–0.01		Bouchoucha et al. (2019)
Algerian coast, Algeria	W	w	4.27	<0.01			0.29				<0.01	4.13	Lounas et al. (2021)
Huelva Ría, Spain	W	d	13.58	0.01			1.87				0.32	44.86	Vicente-Martorell et al. (2009)
Murcia, Spain	W	w					0.15–0.75		0.10–1.76			1.16–8.24	Olmedo et al. (2013)
Bay of Cádiz, Spain	W	d	24.23–84.62	bdl-0.13	bdl-0.06	0.16–0.39	bdl-2.06	2.65–18.63	0.13–0.81	0.31–9.7	bdl-2.51	18.70–55.28	Present study
Aegen Sea, Turkey	F (cages)	w						225.00	6.44			1.08	Erkan and Özden (2007)
Aegen Sea, Turkey	F (cages)	d		0.10–0.40	3.00–4.10	0.80–1.60	0.20–0.30	3.10–5.50	1.30–2.00	0.60–0.90	0.70–1.10	3.40–5.00	Yildiz (2008)
Turkey	F (cages)	d					1.78–2.04	10.22–19.13	0.66–1.23			14.37–19.30	Yigit et al. (2020)
Aegen and Ionian Sea, Greece	F (cages)	d					8.20–14.40					28.82–34.82	Nikolaou et al. (2014)
Aegen Sea, Greece	F (cages)	d				0.13	0.36					2.12	Castritsi-Catharios et al. (2015)
Aegen and Ionian Sea, Greece	F (cages)	w	0.98–2.99	bdl	bdl	0.05–0.15	bdl	bdl-2.77		bdl	bdl	4.29–7.02	Kalantzi et al. (2016)
Heraklion, Greece	F (cages)	w		<0.01							0.01–0.10		Renieri et al., (2019)
Tirana, Albania	F	w		bdl		bdl					0.02		Ozuni et al. (2018)
Adriatic Sea, Croatia	F (cages)	d	<0.01	bdl-0.01	<0.01–0.01	0.24–1.60	0.99–1.26	9.40–40.00	0.45–1.00	bdl	0.02–0.08	12.70–17.50	Žvad Rožič et al. (2014)

(*continued on next page*)

Table 6 (*continued*)

Locality	W/F	d/w	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Caorle Lagoon, Venice, Italy	F (concrete tanks)	w					0.35–2.85	1.30–14.00				3.60–7.60	Carpene et al. (1998)
Leece, Italy	F (intensive tanks)	w		0.44							0.45		Cretì et al. (2010)
Leece, Italy	F (semi-intensive lagoons)	w		0.70							0.52		Cretì et al. (2010)
Ligurian Sea, Italy	F (cages)	d	2.10–7.60				0.80–2.00	5.20–15.20	0.30–1.10			10.70–21.00	Minganti et al. (2010)
Mediterranean Sea, Italy	F	w	0.88–2.83	<0.01		<0.01	0.07–0.14			0.03–0.05	<0.02		Iamiceli et al. (2015)
Corsica, France	F (cages)	w	2.74	<0.01		0.01	0.50	2.47	0.24		0.01	4.34	Marengo et al. (2018)
Tizi Ouzou, Algeria	F (cages)	w	3.52	<0.01			0.52				<0.01	5.02	Lounas et al. (2021)
Ain Temouchent, Algeria	F (raceway)	w	2.40	<0.01			0.45				<0.01	4.78	Lounas et al. (2021)
Tenerife, Spain	F (cages)	w		<0.01			bdl-2.52	1.62–4.64	0.07–0.36	<0.01–0.21	<0.01–0.02	5.16–12.09	Rubio et al. (2011)
Bay of Cádiz, Spain	F (extensive 'estero')	d	16.31–81.99	<0.01–0.35	bdl-0.12	0.12–0.36	bdl-2.37	2.94–25.81	0.14–0.76	0.23–1.14	0.09–4.32	16.66–46.18	Present study
Bay of Cádiz, Spain	F (semi-intensive 'estero')	d	2.62–62.96	bdl-0.05	<0.01–0.09	0.24–2.24	bdl-0.63	5.17–18.61	0.25–3.09	0.46–3.81	bdl-0.84	13.98–32.54	Present study
Vila Nova de Milfontes, Portugal	F (intensive)	w				0.01–0.60	0.49–0.97	2.80–13.00	0.12–0.17	0.01–0.07			Lourenço et al. (2012)

traditionally used was not useful for all species, and that conversion values were ranging from 3.6 to 5.5 depending on the species. Minganti et al. (2010) used 4.31 as factor for *Sparus aurata*.

In cage farming, extra sources of trace metals may result from metal-based antifoulants used (e.g. Cu), as well as from artificial fish diets which are also enriched with various essential metals including Cu, Fe, Zn, Mn, Co, Cr and Mg among others (see Arechavala-Lopez et al., 2013 and reference therein; Nikolaou et al., 2014; Yigit et al., 2020). Although a general comparison showed that values of trace elements in fish from diverse intensive farming systems are also of the same order of magnitude than wild ones (Table 6), detailed comparisons between wild and farmed fish in literature revealed some differences in terms of elements composition (Table 7). For example, higher concentrations of some elements, such as As, were reported in wild populations, while Cu, Fe and Mn showed higher concentrations in farmed fish (Table 7). In some cases, such as Zn and Pb, results vary depending on the studies. The present work and Carpene et al. (1998) found a lower concentration of Zn in semi-intensive and intensive farming systems, while most of studies show lower concentrations in wild populations (Yildiz, 2008; Marengo et al., 2018; Lounas et al., 2021). Contrasting results and discrepancies among studies have been also indicated for some elements by other authors, so the whole elemental profile might be more appropriate than the concentration of an isolated element for comparisons (Arechavala-Lopez et al., 2013).

Regarding extensive farming systems in marsh ponds, trace element signature in muscle of gilthead seabream from these systems were similar to those detected in wild conspecifics, being both different from element signature found in seabream from semi-intensive ponds (Tables 3 and 5). In any case, the values measured in the present study for wild-caught, extensive cultured and semi-intensive cultured gilthead seabream specimens are below the limit values provided by national and international regulations for all the elements except for As (Table 8). As is present in fish under various chemical forms and the most abundant one is the non-toxic organic arsenic (arsenobetaine) (Abadi et al., 2015; Micheline et al., 2019). The toxic forms are mainly As(III) and As(V). The current guidelines for As exposure are provided only for inorganic As (Table 8), since the organic forms of arsenic has no toxicological concern (Micheline et al., 2019). The relatively high values obtained for As in the present study are also reported for other fish species from the Bay of Cádiz (Guerra-García et al., 2023). But these values do not reflect the health hazard because the toxic fraction (inorganic As) in fish ranges between 0.02 and 11% of the total As (Muñoz et al., 2000). In this sense, Kalantzi et al. (2016) estimate human risk due to As based on inorganic fraction calculated as 10% of total As. According to this, values of inorganic As in the present study would be, in general terms, below the limits proposed (Table 8). Despite of European regulations have not established limits of As for fish and other foodstuff in human consumption yet (Table 8), Regulation No 1006/2015 (EC European Commission Regulation, 2015b) provided limits of 0.1–0.30 of inorganic As (addition of As (III) and As (V)) in rice and derivates since rice is an important ingredient in a variety of food for children. Therefore, the health risk arising from the consumption of the studied extensive and semi-intensive farmed fish due to metals content is minimal and there would be only concerns about As (not EC limits available) and Hg (not measured in the present study) which would need further investigation.

4.3. *Promoting extensive cultures in 'esteros'*

Sustainable aquaculture is a priority in the current economic and social context (Abdou et al., 2017), and systems for aquaculture production must be adapted to improve environmental performance and decrease energy consumption (D'Orbcastel et al., 2009). The increase in aquaculture production to meet consumer demand has resulted in a number of farmed species entering the marketplace in the last decades, which includes gilthead seabream *S. aurata* (Morrison et al., 2007). There is no market integration between wild and farmed gilthead

Table 7
Comparison of trace metals between wild (W) and farmed (Fi: intensive, Fe: extensive) *Sparus aurata* from the literature reviewed.

Locality	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Aegen Sea, Turkey		n.s.	n.s	n.s.	n.s.	n.s.	n.s.	n.s.	Fi > W	Fi > W	Yildiz (2008)
Heraklion, Greece		n.s.							W > Fi		Renieri et al., (2019)
Adriatic Sea, Croatia	n.s.	n.s.	W > Fi	n.s.	n.s.	Fi > W	n.s.	n.s.	n.s	n.s	Žvad Rožič et al. (2014)
Caorle Lagoon, Venice, Italy					n.s.	n.s.				W > Fi	Carpene et al. (1998)
Ligurian Sea, Italy	W > Fi				n.s.	n.s.	n.s.			n.s	Minganti et al. (2010)
Corsica, France	W > Fi	n.s.		W > Fi	Fi > W	n.s.	Fi > W		n.s	Fi > W	Marengo et al. (2018)
Algerian coast, Algeria	W > Fi	Fi > W			Fi > W				W > Fi	Fi > W	Lounas et al. (2021)
Bay of Cádiz, Spain	W > Fe > Fi	n.s.	n.s	Fi>(W=Fe)	n.s.	Fi>(W=Fe)	Fi>(W=Fe)	n.s.	n.s	(W=Fe)>Fi	Present study

Table 8
Limits (mg/kg ww) for some trace elements in fish for human consumption recommended by different national and international regulations. Average values (mg/kg ww) obtained in the present study for *Sparus aurata* are included for comparison (Mean dry weight were converted in wet weight values dividing by 4.31 for comparison, according to Minganti et al., 2010). *Reference limits for As are provided for inorganic As (Micheline et al., 2019). Inorganic fraction of As for the present study has been estimated as 10% of total As according to Kalantzi et al. (2016) (total As in included in parentheses). Limits for Hg were not included since Hg was not measured in the present study. Modified from Guerra-García et al. (2023).

	As*	Cd	Cr	Cu	Pb	Zn	Reference
National and International Regulations:							
Spanish legislation		1		20	2		BOE (1991, 2006)
Croatian legislation	2	0.05–0.3			0.3		Ordinance (2008); Žvab Rožič et al. (2014)
Turkish legislation		0.1		20	1	50	Anonymous (1996); Dural et al., (2007); Korkmaz et al., (2017)
Ministry of Agriculture, Fisheries and Food, UK		0.2		20	2	50	MAFF, 2000; Korkmaz et al., (2017)
European Commission		0.05–0.25			0.2–0.4		EC European Commission Regulation (2001, 2006, 2008, 2014, 2015a); Lozano et al. (2017)
United States Environmental Protection Agency		2	8	120	4	120	USEPA, 2011; Kumar et al. (2021)
Government of Canada	3.5				0.5		Government of Canada, 2020
Australia New Zealand Food Authority	2				0.5		Australian Government, 2021
China National Standards	0.5	0.1	2	50	0.5		CNSMD (2001)
Brazilian authorities		1		30	2	50	Joyeux et al. (2004)
Food Safety and Standards Authority of India		0.3			0.3		FSSAI Food Safety Standards Authority of India, 2015; Kumar et al. (2021)
Malaysian Food Regulation		1		30	2	100	MFR (1985); Kumar et al. (2021)
South African Department of Health	3	1			0.5		Bosch et al. (2016)
World Health Organization		1	50	30	2	100	WHO (1989); Kumar et al. (2021)
Food and Agriculture Organization of the United Nations (FAO)	0.1–6	0.05–2	1	10–100	0.5–6	30–150	Nauen (1983)
***Sparus aurata* from Bay of Cádiz (present study):**							
Wild (W)	1.27 (12.72)	0.01	0.06	0.10	0.25	7.39	
Extensive (E)	0.81 (8.09)	0.01	0.06	0.12	0.23	7.09	
Semi-intensive (S)	0.31 (3.12)	<0.01	0.21	0.05	0.08	4.64	

seabream which implies that capture fisheries are not affected by increases in the aquaculture production of this species (Bjørndal and Guillen, 2017). Indeed, gilthead seabream has become one of the most important marine species in the Mediterranean aquaculture sector, with largest productions coming from intensive farming located both on land and mainly in floating cages at sea. Although farming facilities are designed to optimize growth and health, intensive farming systems could cause welfare problems such as stress, poor condition of the fish and decrease in product quality (Ashley, 2007). The high densities of intensive production have, therefore, concerns which warrant further research and new alternatives are needed to improve fish welfare (Matos et al., 2017). In this sense, unlike intensive systems, extensive farming in marsh ponds constitute adequate natural habitats which ensures positive effects on fish welfare (e.g. natural preys, use of space, etc.) but also provide a quality fish product. Although sensory characteristics of farmed products seem to meet the quality requirements expected by consumers, and may be even more appreciated than wild fish in blind conditions (Claret et al., 2016), farmed fish have a less positive image among consumers than their respective wild-caught equivalents. Indeed, European consumers believe that wild fish have a higher quality, while farmed fish are superior in terms of control, price and availability (López-Mas et al., 2021). The present study shows that wild-caught and extensive cultured gilthead seabream have many similarities.

Although the largest amount of gilthead seabream production in European market comes from intensive production, there is still a large production from land-based semi-intensive and extensive systems, mainly from Spain and Portugal (Matos et al., 2017). With the decline of salt industry over the 20th century, many abandoned salt works in southern Spain were partially adapted to extensive fish farming and some of them were completely transformed for semi-intensive fish monoculture. The farming activity has contributed to maintain this particular and valuable landscape; however, the low profitability and the progressive abandoning of ponds are primary threats for the prevalence of this area (Yúfera and Arias, 2010). Some initiatives, such as the SEACASE (Sustainable extensive and semi-intensive coastal aquaculture in Southern Europe) project, funded by the European Commision (FP6), have emerged to improve the profitability by increasing the water surface useful for fish farming through the creation of new marsh ponds while maintaining the sustainability and environmental quality of these areas. Indeed, from the beginning of salt industry decline in Bay of Cádiz, the extensive fish production has increased progressively from 30 kg per ha in the 1940′ up to 300 kg per ha in the change to XXI century (Yúfera and Arias, 2010). However, these authors point out that producers must face several problems: (i) the increase of fish production in extensive and semi-intensive cultures have been accompanied by an increase of aquatic bird's populations, and it is estimated that 15–30% of fish production is lost due to bird predation, and (ii) the role of these extensive farms in maintaining the environment is not well recognized by the society. Conversely, other ways to improve efficiency of extensive and semi-intensive cultures are being developed. The aquaculture farm-wetland complex of Veta la Palma (SW Spain) is an example of a currently viable land-based IMTA (Integrate Multitrofic Aquaculture) which combine the semi-intensive production of sea bass, *Dicentrarchus labrax* (Linnnaeus, 1758) in grow-out fish ponds with the extensive production of Mugilidae and Palaemonidae naturally recruited in multitrophic polyculture lagoons (see Fernández-Rodríguez et al., 2018 for details). Additionally, farmer associations in southern Spain have launched the quality seal "fish of estero" as a trade mark for fish cultured in these ecosystems. This fish is sold in local markets with intermediate prizes between the wild ones (the more expensive) and those coming from the net cages aquaculture facilities open sea (the cheapest).

To conclude, the present study reveals that both extensive and semi-intensive 'esteros' offer a promising environment for aquaculture farming. Particularly, the extensive system provides a fish product more similar to the wild conspecifics, with similarities in trophic niche, and concentrations of trace metals below the legal limits. For all these reasons, marsh ponds, especially extensive ones, should be promote as environmentally sustainable ecosystems to contribute to the diversification of aquaculture products. The implementation of innovative strategies will help to increase responsibility, sustainability and profitability in marsh ponds, and further economic support of funding agencies and national budgets are specially needed, as well as marketing initiatives to promote fish products from marsh ponds.

CRediT authorship contribution statement

J.M. Guerra-García: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft. **S. Calero-Cano:** Investigation, Data curation, Methodology, Writing – review & editing. **I. Donázar-Aramendía:** Investigation, Methodology, Writing – review & editing. **Giráldez I:** Investigation, Methodology, Writing – review & editing. **Morales E:** Investigation, Methodology, Writing – review & editing. **P. Arechavala-Lopez:** Investigation, Methodology, Writing – review & editing. **J.L. Cervera-Currado:** Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2023.106007.

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