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TRACE ELEMENT SIGNATURE ON OTOLITHS AND SCALES FOR THE DETECTION OF ESCAPED SEABASS AND GILTHEAD SEABREAM FROM MEDITERRANEAN FISH FARMS

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El presente trabajo ha sido llevado a cabo en su totalidad en el Departamento de Ciencias Marinas y Biología Aplicada (DCMBA) y el Centro de Investigaciones Marinas (CIMAR) de la Universidad de Alicante (UA), España, bajo la dirección del Dr. Pablo J. Sánchez Jeréz (UA) y de la Dr. Carmen María Hernández Cruz (ULPGC).

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A Miguel

ABSTRACT

Significant differences in trace element signature of scales and otoliths have been found between wild and farmed *Sparus aurata* and *Dicentrarchus labrax* in the southwestern Mediterranean. Furthermore, trace element composition in *S. aurata* scales gave away significant differences between two different fish farms subject to this work. The goal of the present study was to analyze trace element signature in scales and otoliths of farmed and wild European seabass (*Dicentrarchus labrax*, Linnaeus 1758) and gilthead seabream (*Sparus aurata*, Linnaeus 1758), two commercially important species in the Mediterranean, and test if it is possible to use trace element signatures in those two tissues as a way to identify escaped farmed fish in the wild.

Escapes from sea cages happen for almost all currently sea cage farmed fishes, including the seabass and seabream subject to this study. There are several ecological impacts of escapees on wild populations, and the extent of these impacts has not yet been accurately evaluated. The ability to tell apart farmed from wild fish makes it possible to detect escapees in the natural populations without artificial tagging, which in turn helps to better understand the behaviour of the escapees in the natural environment. This is one of the key steps to take to mitigate the effects of escapes from sea cage farms.

Keywords: sea cages, escapes, otoliths, scales, ICP-MS, seabass, seabream

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1. INTRODUCTION

1.1. Seabass and gilthead seabream life history

European seabass (*Dicentrarchus labrax*, Linnaeus 1758) and gilthead seabream (*Sparus aurata*, Linnaeus 1758) occur naturally in the Mediterranean. *D. labrax* also inhabits the Black Sea and the north Atlantic, from Norway to Morocco and the Canaries. *S. aurata* is absent from the Black Sea and in the north Atlantic it ranges from Great Britain to Cabo Verde. Both are demersal species that dwell in the littoral zone, on a variety of bottoms, although seabass prefers slightly deeper waters than seabream. Seabass depth range is between 10 – 100 m (Frimodt, 1995), rather to 10 m than to 100 m (Lloris, 2002). Seabream lives between 1 - 150 m depth (Muus and Nielsen, 1999), however it is mostly found in the top 30 m zone (Lloris, 2005). Seabass appears to be more gregarious than seabream (Frimodt, 1995), and the adults are also more piscivorous (Kottelat and Freyhof, 2007) than seabream, who prefer shellfish (Bauchot and Hureau, 1990). Both species also consume crustaceans (Tortonese *et al.* 1986). Seabass and seabream are protandrous hermaphrodites that produce pelagic eggs (Muus and Nielsen, 1999).

Seabass and seabream make up most of the fish produced in sea cages in the Mediterranean, with a production of 84593 tons of seabream and 57893 tons of seabass in 2007 (FAO 2007).

1.2. Sea cage aquaculture and ecological impact of escaped fish

Europe has become a world leader in sea cage aquaculture, producing over a million tons of fish per year (FAO 2007). The ecological impacts of such activity include release of nutrients (Islam 2005, Karakassis *et al.*, 2000, 2005), interactions with wild fauna (Dimitrou *et al.*, 2007, Krkošek *et al.*, 2007) and deposition of polluting matter (deBruyn *et al.*, 2006, Sather *et al.* 2006) among other impacts. Escapes from sea cages happen for almost all currently cage farmed fishes, such as Atlantic salmon, cod, arctic charr, halibut, seabass, gilthead seabream, and meagre. The escape of farmed fish from sea cage aquaculture poses several threats over the local populations, such as: transfer of pathogens and parasites between farmed and wild fish (Diamant *et al.* 2000 and 2005; Sepúlveda *et al.* 2004; Morton *et al.*, 2005; Krkošek *et al.*, 2005), competition for resources (Toledo *et al.*, 2008), and negative interbreeding with wild members of the same species (Fleming *et al.* 2000; McGinnity *et al.*, 2003; Hindar *et al.*, 2006).

Despite the fact that seabass and seabream are native species to the Mediterranean, several generations of farmed fish have produced populations that are dramatically different from wild populations of the same species (Youngson *et al.*, 2003). Skaala *et al.*, 1990 and Volpe *et al.*, 2001 have documented that farmed salmon can interbreed with wild salmon, introducing genetic changes in wild populations.

Genetic differentiation exists between wild stocks of seabass (Allegrucci *et al.*, 1997; Lemaire *et al.*, 2000) and between wild and culture stocks (ICES WGEIM, 2006). Furthermore, farmed seabass are reported to produce viable sperm and eggs in cages (ICES WGEIM, 2006). Hence opportunities for interbreeding are highly probable, even if no interbreeding has yet been reported (Bahri-Sfar *et al.*, 2004). On the other hand, breeding of intentionally released farmed seabream with the wild population has been reported one year after they had been released 15 km off the coast in southern Spain (Sánchez-Lamadrid, 2004). Released fish were mature and were caught in shoals, mixed with wild conspecifics. They showed the same spawning behavior as wild specimens after one year in the natural environment, strongly suggesting gene flows between escaped and wild seabream. All these threats have been documented, but their actual extent is yet to be accurately assessed. It is necessary to increase the effort to evaluate the risks and in the meanwhile, maximize the precautionary measures (Naylor *et al.*, 2005; ICES WGEIM, 2006; IUCN, 2007; Thorstad *et al.*, 2008).

There is a variety of factors that allow fish to escape, such as technical or operational failures, cage wear out, extreme weather conditions, etc. (Sánchez-Jerez *et al.*, 2007; Hansen *et al.* 2008). Nowadays, it remains impossible to completely avoid escapes from fish farms (Naylor *et al.*, 2005). Under several circumstances, such as extreme weather or operational accidents, escapes will occur. Hence, it is important to develop cost-effective tools for identifying escaped fish in order to assess the extent and consequences of escapes. Further knowledge of fish behavior and dispersal after escaping can be directly used for

assessing the possibilities of recapture and the development of recapture technologies.

1.3. Distinguishing between farmed and wild fish

Several methods have been used in the past to tell apart wild from farmed fish, including morphology (Youngson and Hay, 1996), presence of synthetic pigments from fish feed (Turujman *et al.*, 1997) or physical characteristics of scales (Hillivirta *et al.*, 1998), due to the fact that farmed fish grow faster than wild fish (Lund and Hansen, 1991). Genetic techniques have also been used for this purpose, and they proved to be very useful for stock identification. The drawback, however, is that genetic studies are more expensive and are not able to provide information about the environment where the fish has been living (Beacham *et al.*, 1995).

External tags have inherent low recapture rates, with reported values as low as 1.9% (Hansen and Jacobsen 2003), making artificial tagging cost-ineffective and low rewarding. Advanced tagging methods involving acoustic and radio detection, together with associated satellite telemetry, have shown promising results in determining fish movements in pelagic species, such as bluefin tuna. (Åkesson et al 2002). Nevertheless, these techniques have limited success rates on demersal species, such as the gilthead seabream, subject to this study.

As a solution to artificial tagging, the use of natural tags to identify an escaped fish appears to be the logical step to take in environmental impact studies of escaped

fish from sea cages. Several fish structures have been used for this purpose, such as otoliths, scales, spines and eye lenses (Radtke and Shepherd 1991; Coutant and Chen, 1993; Dove and Kingsford, 1998; Wells *et al.*, 2000).

Stock discrimination based on trace element signature is built on the hypothesis that fish living in different water bodies will incorporate trace elements from the environment into their calcified structures, forming a unique chemical signature that will reflect the length of time that a fish has inhabited a particular water body (Elsdon and Gillanders, 2003).

1.4. Composition and structure of scales and otoliths

Fish scales and otoliths are important tools for understanding the life of fish and fish populations. As fish grow, scales and otoliths also grow proportionally, with *circuli* laid down much like the growth rings of a tree. As growth slows down during the winter, the *circuli* of scales and otoliths bunch closer together and form darker bands known as *annuli* (figures 1 and 2).

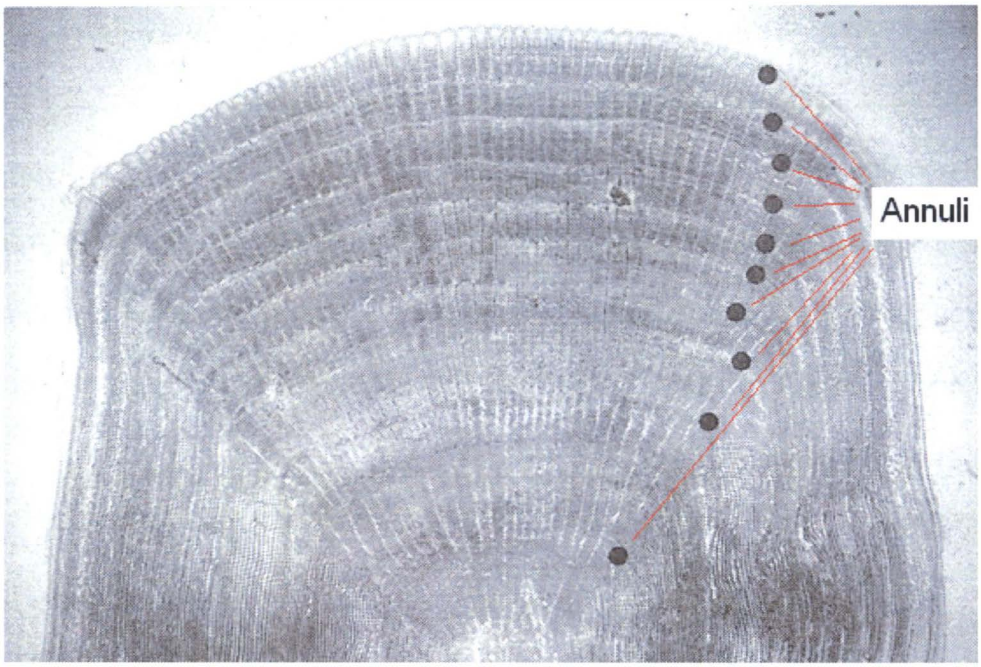

Figure 1. Structure of a ten year old striped seabass scale (*Morone saxatilis*) (source: John Boardman, MA Division of Marine Fisheries).

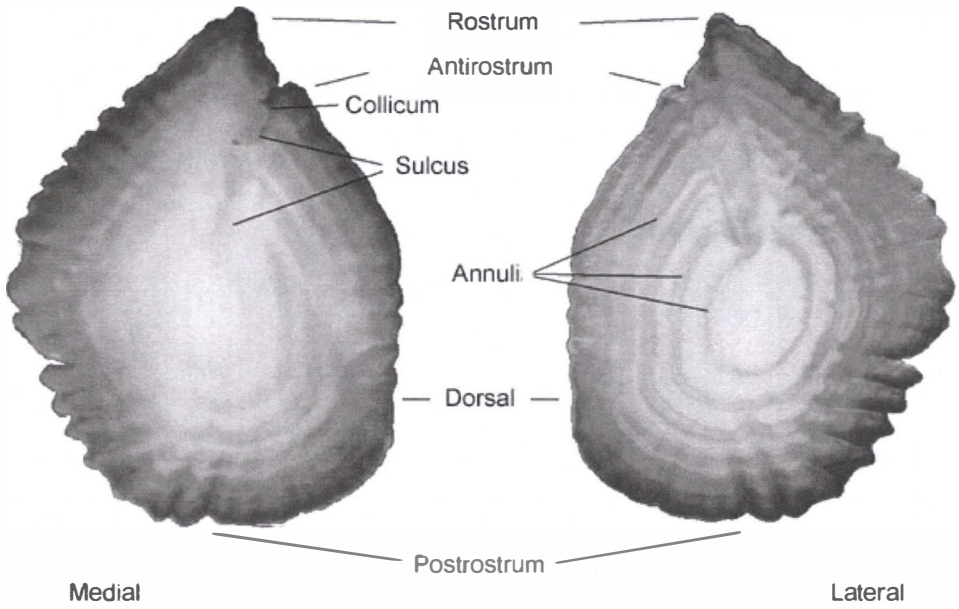

Figure 2. Structure of a Chilean cardinal fish (*Epigonus crassicaudus*) otolith (source: Luis A. Cubillos).

These *circuli* and *annuli* record age and growth patterns of the fish in great detail. Virtually the entire growth history of a fish is recorded in its scales and otoliths (Gauldie *et al.*, 1980).

1.4.1. Scales

Scales are protective dermal plates which grow on the skin of most fish and serve to protect, color, and support the body. They are mainly composed of layers of hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$) and calcium carbonate ($CaCO_3$). The information that scales contain in its structure can be used for age validation and also to detect environmental markers, heavy metal bioaccumulation or other chemical signs (Coutant and Chen, 1993; Wells *et al.*, 2000, 2003; Gillanders, 2001). Another important characteristic of scales is that they are easily removed without harming the fish (Muhlfeld *et al.*, 2005).

1.4.2. Otoliths

Otoliths are compact, acellular structures made of protein and calcium carbonate ($CaCO_3$). They are found in the head of most fishes which provide the fish with a sense of balance and orientation, and they also aid in hearing (Brown and Wellings, 1969; Campana and Neilson, 1985). Finfish have three pairs of otoliths: the *sagittae*, the *lapilli* and the *asterisci* (figure 3). The *sagittae* are the largest,

found just behind the eyes and approximately level with them vertically. The *lapilli* and *asterisci* (smallest of the three) are located within the semicircular canals (Secor *et al.*, 1991).

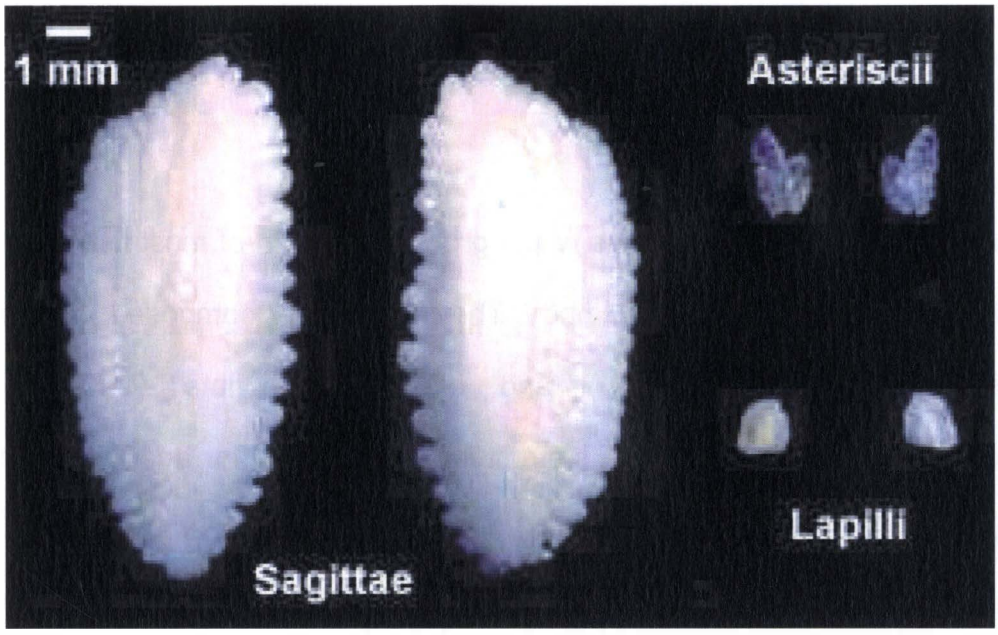

Figure 3. Three pairs of cod (*Gadus morhua*) otoliths: *sagittae*, *asteriscii* and *lapilli* (source: Steven E. Campana).

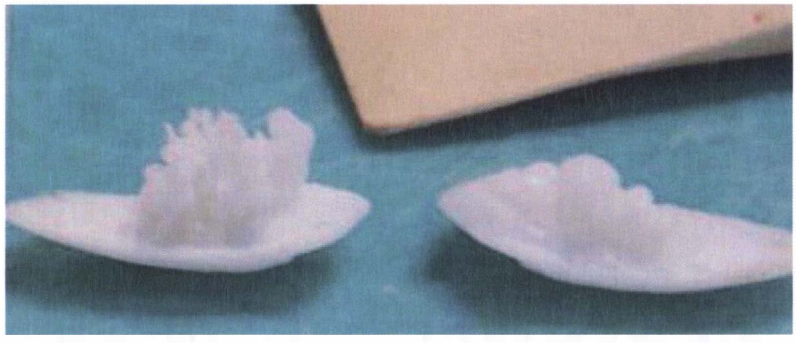

Figure 4. Seabass *sagittae* otoliths showing the substitution of aragonite by vaterite (in the otolith in the left), which resembles staghorn coral (source: Jonathan P. Williams).

The $CaCO_3$ is usually deposited as aragonite in the *sagittae* and *lapilli* and as *vaterite* (a polymorph of aragonite) in the *asterisci*. However, in a small percentage of the seabass samples used in this study, part of the aragonite matrix had been partially replaced by *vaterite*, which is usually only found in the *asteriscii* (Thresher, 1999). This substitution is thought to be associated with stressful situations, like stocking (in farmed fish) (Falini *et al.*, 2005), but the reason behind this substitution remains unknown. In the present study, those substitutions were exclusively found in wild seabass, but this was not considered to influence trace element deposition, since the matrix of both polymorphs has the same separation between atoms (Falini *et al.*, 2005).

1.5. Deposition of trace elements in scales and otoliths

Trace elements present in the water or the diet enter the fish via the gills or the gut epithelium, pass to the blood plasma and are either directly incorporated to the scales or passed to the endolymph where they finally crystallize and deposit in the otolith matrix (Campana 1999). Several factors influence trace element deposition, such as salinity (Elsdon and Gillanders, 2002; Kraus and Secor, 2004; Martin *et al.* 2004), temperature (Tzeng, 1994; Arai *et al.*, 2002), growth rate (Kalish, 1989) and the developmental and reproductive stages (Kalish 1990; Clarke and Friedland 2004). Hence trace element profile is likely to be unique to a given population that inhabits one given location. For this reason, trace element analysis has been used on many different species to determine fish origin (Lo-Yat *et al.*, 2005; Patterson

and Kingsford, 2005), migrations (Elsdon and Gillanders, 2003), distinguish between farmed and wild fish (Adey, 2007) and population structure (Gao *et al.*, 2005). Wild populations of seabass and seabream in the studied zone, the southwestern Mediterranean, are known to roam between different zones, and for this reason it is usually difficult to find big differences in trace element signatures among different populations of wild fish (Gillanders *et al.*, 2001). Nevertheless, aquaculture creates a special situation in which the normally roaming species become fixed in one specific location with also unique environmental conditions. Under such circumstances, differences in trace element composition are likely to appear between the studied fish (Lo-Yat *et al.*, 2005; Patterson and Kingsford, 2005) as they appear bound to one specific location.

The calcified tissues used in the present work do not incorporate trace elements in the same way. Otoliths are detached from the watery environment of the fish and only incorporate trace elements that are present in the endolymphatic fluid that bathes them, whereas scales incorporate trace elements directly from the blood plasma (Wells *et al.*, 2000).

2. MATERIAL AND METHODS

2.1. Location and sample collection

The present study was carried out in the southeast coast of Spain, in two different locations within the province of Alicante: Altea and Guardamar del Segura, where the farms subject to this study are located (see figure 5).

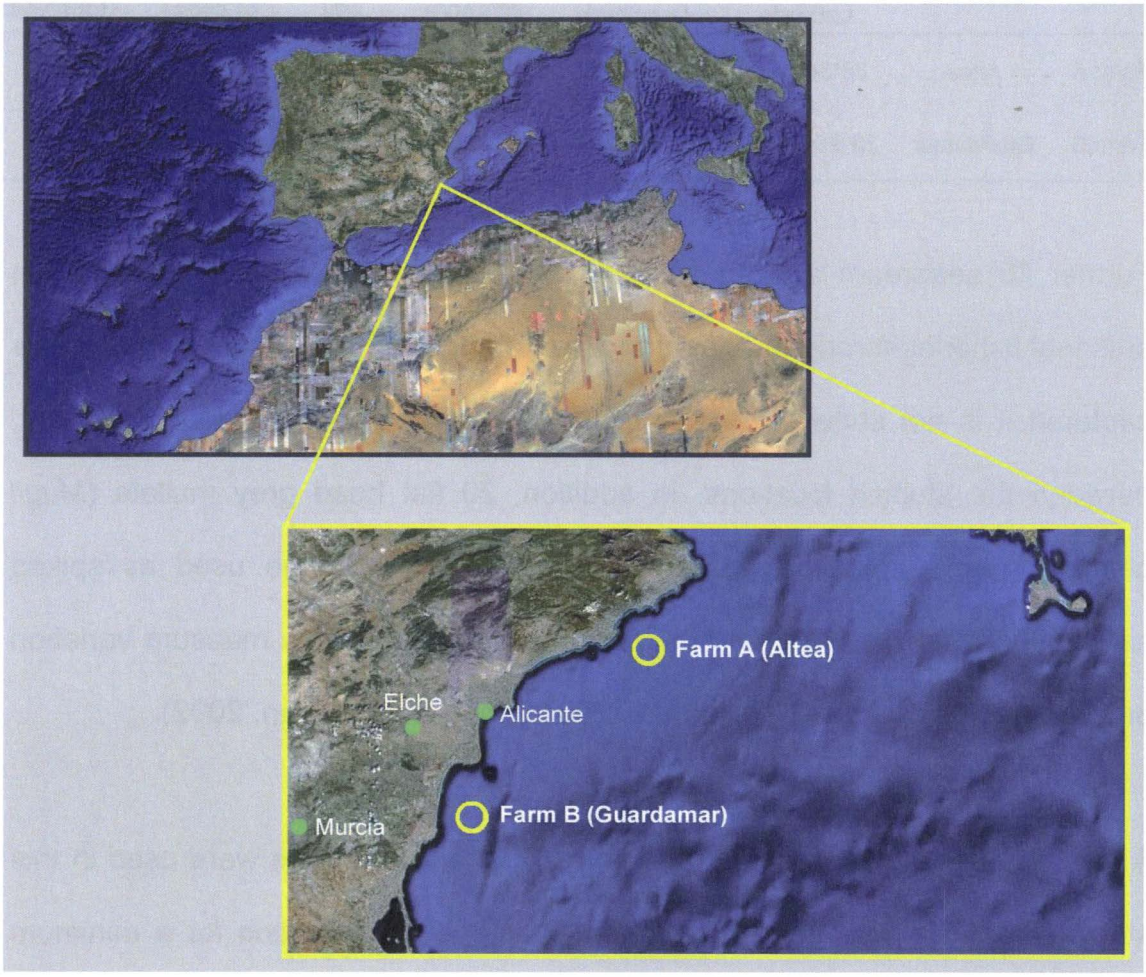

Figure 5. Locations where the study took place (source: Google Earth® image).

The farm A is located in Altea (latitude 38º34'27"N, longitude 0º02'68"W) 2.8 km offshore, where the average depth is 34 m. The annual production is roughly

500 tons of *S. aurata* and *D. labrax*. The farm B is located in Guardamar del Segura (lat. 38° 6'39.12"N, long. 0°36'52.07"W), 3. 7 km offshore, where the bottom is 22.6 m deep. The farm produces around 1200 tons of seabass and seabream every year. Both farms belong to the same company: "Grupo Culmarex". 15 fish of each species were taken from each farm. Details of both farms can be seen in table I.

Table I. Characteristics of the farms subject to the study.

Group	Location	Position		Km offshore	Depth (m)	Number of cages	Production (tons/year)
		Latitude	Longitude				
Farm A	Altea	38°34'27"N	0°02'68"W	2.8	34	12	500
Farm B	Guardamar	38°6'39.12"N	0°36'52.07"W	3.7	22.6	24	1200

Further 15 seabream and 15 seabass were collected as control group from artisanal fisheries throughout the province. The location where the wild fish was captured it is not shown in the map, but the fishermen work near the coast, between the studied locations. In addition, 20 flat head grey mullets (*Mugil cephalus*) were collected near the Farm B location to be used as spiked samples for the ICP-MS analysis in order to detect possible measure variation or inaccuracies over time (Gillanders, 2001; Gordon and Swan, 2002).

A total number of 45 seabass, 45 seabream and 20 mugilids were used in this study. Every fish was deep frozen immediately after collection for a minimum period of 48 hours to prevent bacterial degradation. For each individual, total length (TL), fork length (FL) and standard length (SL), all rounded down to the nearest 0.1 cm were measured prior to the collection of otoliths and scales.

2.2. Scales and otoliths collection

All the scale samples, including the spiked ones, were specifically collected for this study. It is known that the sampling method can affect trace element concentrations (Adey, 2007). Hence 10 to 20 scales from the left hand side of the fish, 2-3 rows above the lateral line (figure 6) were removed using one disposable plastic knife per fish.

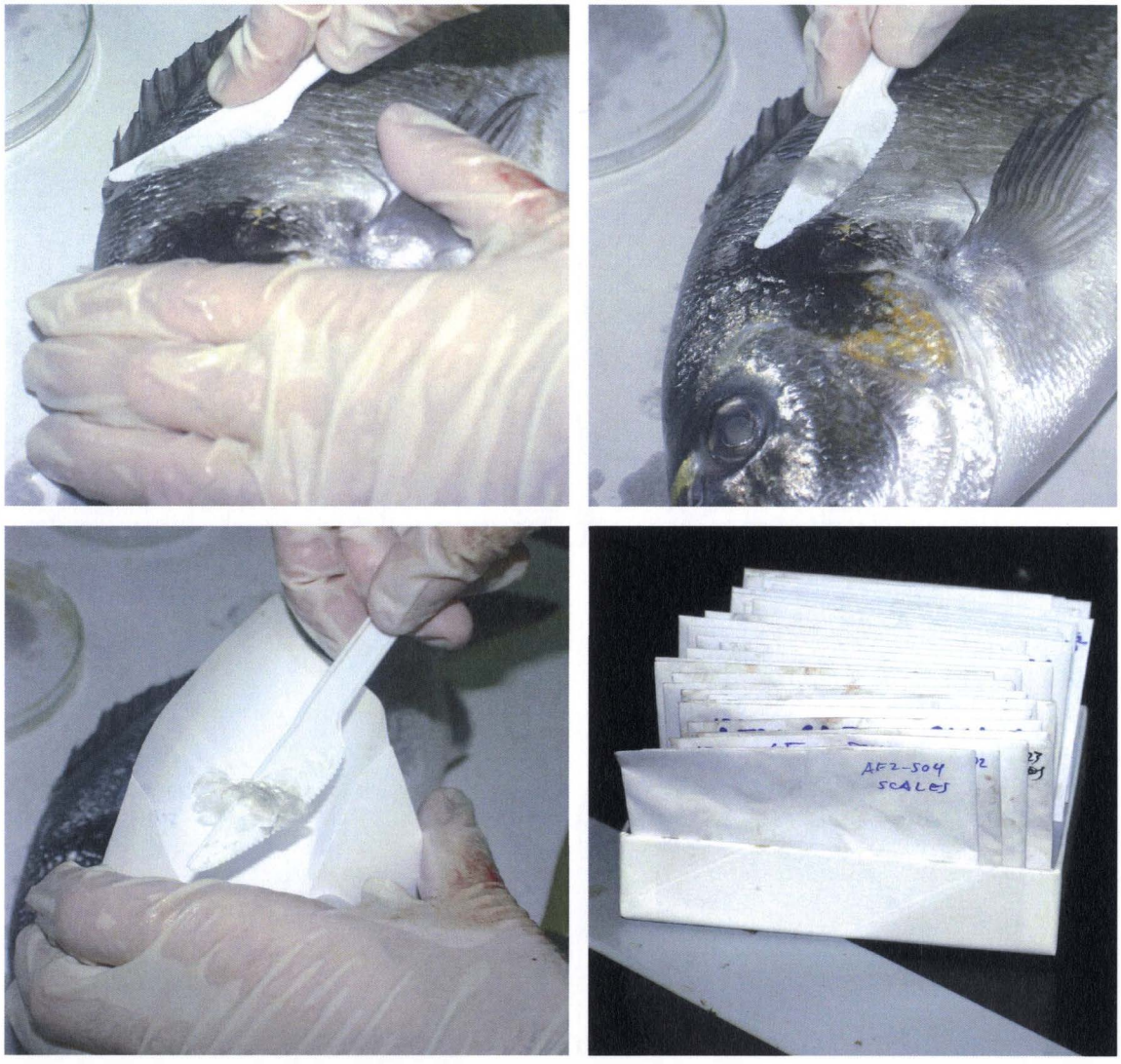

Figure 6. Seabream scale collection using a disposable plastic knife and paper envelopes.

The plastic knife was rinsed with 18 ohm reverse osmosis water before touching the fish to reduce even more any possible influence of the knife on the trace element profile. The scales were stored in paper envelopes upon further procedure.

For the otolith collection, the dissection was carried out using a stainless knife, carefully rinsed with 18 ohm reverse osmosis water between individuals.

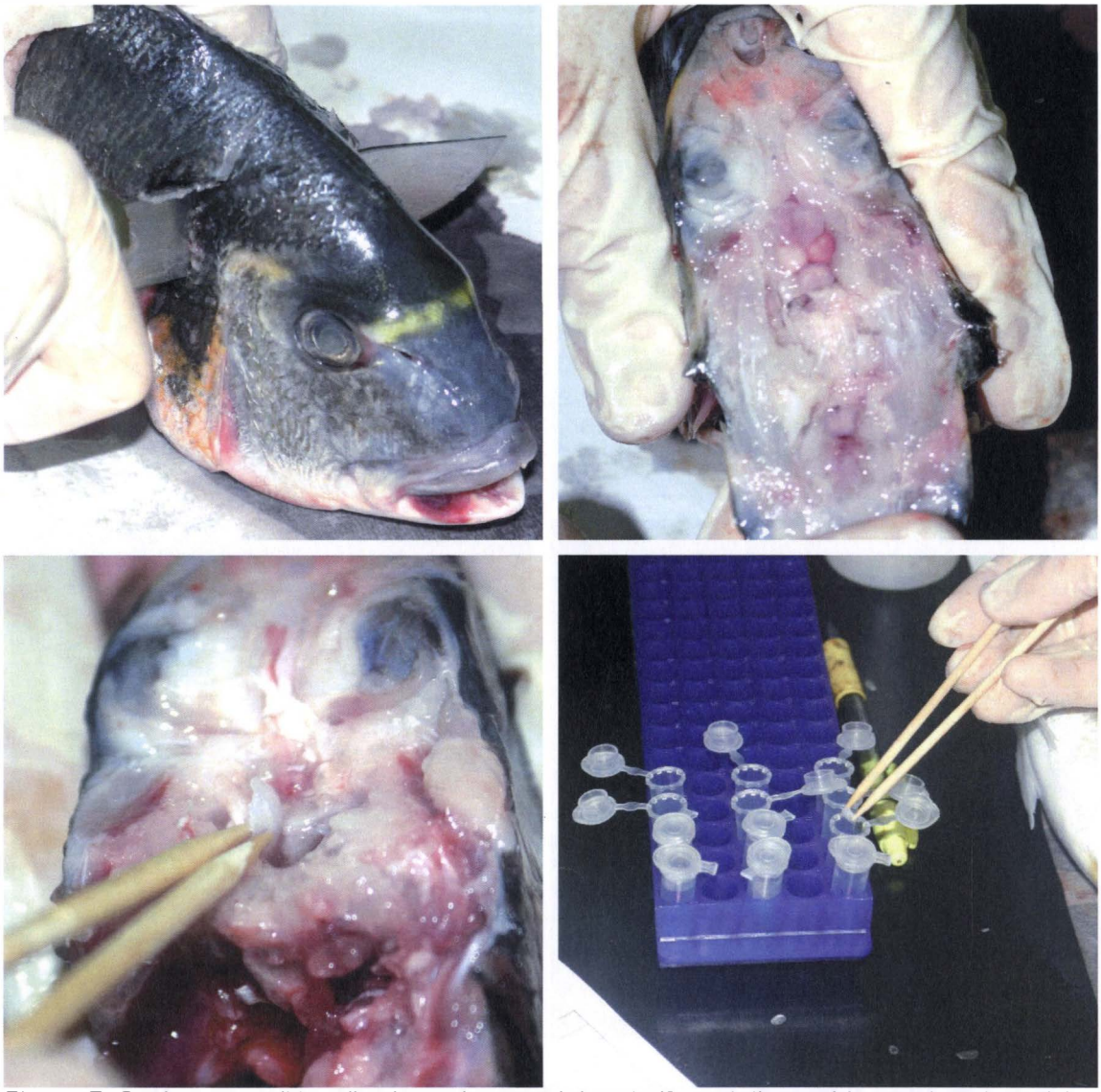

Figure 7. Seabream otolith collection using a stainless knife and disposable wooden chopsticks.

Disposable wooden chopsticks were used to remove the sagittae pair of otoliths (figure 7). A different pair of chopsticks rinsed with 18 ohm osmosis water was used for each fish. The otoliths were rinsed with 18 ohm reverse osmosis water before storage in 1.5 mL test tubes. All the otoliths were specifically collected for this study.

2.3. Equipment cleaning procedure

All the equipment, reusable as well as disposable, was cleaned with the following protocol, based on the one used by Adey, 2007, prior to its use: First, it was soaked for 4 hours with a chelating agent (EDTA 0.5%) to prevent possible metal contaminations, and then moved to an acid wash in superpure HNO_3 10% for 24 hours. Then, all equipment was thoroughly rinsed with 18 ohm reverse osmosis water and left to soak in the 18 ohm osmosis water overnight and then left to dry inside the fume cupboard.

Only HNO_3 UpA (ultra pure acid) 69% and ultra pure H_2O_2 33% were used in all procedures. HNO_3 UpA has less than 10 ng/Kg metallic impurities. H_2O_2 metallic impurities were in a concentration less than 50 ng/g. These impurities have no effect on trace element results, because all the impurities are far below the ICP-MS detection level. However, background levels were assessed using digestion blanks and those levels were subtracted from sample results. 18 ohm reverse osmosis ultra pure water was used throughout the whole study.

2.4. Scale procedure

2.4.1. Scale cleaning procedure

Due to the thinness of the scales, they are unfit for an acid wash (Wells *et al.*, 2000). Hence, the scales were sonicated in H_2O_2 3% for 5 minutes, then rinsed thoroughly with 18 ohm reverse osmosis water and left to dry completely inside the fume cupboard for a period over 48 hours. The scales were handled with plastic tweezers cleaned with the general equipment cleaning procedure, and no metal came in contact with the scales at any point of the procedure. This protocol was based on the one used by Wells *et al.* 2003a.

The dry scales where weighed in a precision weight rounded down to the nearest 0.01 mg. Whole sample weight was written down and subsamples of around 0.03 ± 0.0001 grams were prepared for the ICP-MS analysis. These subsamples were put into new 1.5 mL test tubes which had had previously underwent the equipment cleaning procedure.

2.4.2. Scale digestion

6 mL of HNO_3 UpA (5%) and 2 mL of H_2O_2 (3%) was added to each test tube. The tubes were, then, taken to the microwave (Pender and Griffin, 1996; Gillanders *et al.*, 2001). The samples had their temperature raised from 27ºC (room temperature) to 190ºC in 10 minutes time. That temperature of 190ºC

was maintained for 20 minutes. 20 digestion blanks were prepared alongside samples (which included the spiked samples). The digestion was completely achieved in all samples. The resulting solutions were diluted to a volume of 25 mL using 18 ohm reverse osmosis ultra pure water.

4.2.3. Scale spiked samples preparation

The digested scales of the mullets were mixed homogeneously to form a stock solution, from which 20 samples were taken in order to analyze 2 stock solution samples for every 9 study samples, apart from the 2 digestion blanks to be analyzed with each run (Gillanders, 2001) with the ICP-MS.

4.3. Otolith procedure

4.3.1. Otolith cleaning procedure

This protocol was necessary to remove the external layer of the otolith and prevent artifacts, due to possible contamination from otolith handling. This protocol was also based on the one used by Wells *et al.*, 2003a. All the otoliths were submerged in 18 ohm reverse osmosis water in order to rehydrate the otolith covering matter for 24 hours. Afterwards, each otolith was dipped in H_2O_2 3% for 5 minutes, then in HNO_3 1% for 20 seconds and finally thoroughly rinsed in 18 ohm reverse osmosis water. The otoliths were then left to dry completely

for at least 24 hours. Once they were completely dry, all the otoliths had their weight measured rounded down to the nearest 0.01 mg, and finally stored in 10 mL test tubes.

4.3.2. Otolith spiked samples preparation

The *M. cephalus* otoliths underwent the previously explained procedure. Then, they were all ground together to a fine powder with a china hand grinder and 0.06 ± 0.0001 grams of otolith powder were transferred to the test tubes. The whole process took place inside the fume cupboard.

4.3.3. Otolith digestion

All the study otoliths and the spiked samples were submerged in 2 mL ultra pure HNO_3 10 % (Lo-Yat *et al.* 2005) and left overnight to achieve complete digestion. Then, 8 mL of 18 ohm reverse osmosis water was added to each tube to raise the volume to 10 mL in each tube and drop the acid concentration to 2% in every tube. The digested samples were then analyzed with the ICP-MS. Digestion white samples were prepared alongside otolith digestion samples. As with scales, 9 study samples, 2 spiked otolith samples and 2 digestion blanks were analyzed in each run of the ICP-MS.

2.6. ICP-MS analysis

Inductively coupled plasma mass spectrometry, or ICP-MS, is the routine method of choice for trace elements determination in environmental studies (Campana *et al.*, 1994; Campana and Gagne, 1995; Wells *et al.*, 2000; Flem *et al.*, 2005). ICP-MS allows simultaneous determination of most elements within the periodic table with limits of detection below one part per billion (10^{12}) (Miller and Miller, 1998). Solution based ICP-MS was chosen for this study due to its greater precision compared with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (Campana *et al.* 1994). Each sample was measured three times, obtaining a mean value and a standard deviation per element and sample.

ICP-MS consists of six stages: sample introduction, argon plasma ion source, cones for sampling ions from the plasma, ion lenses for selecting and focusing ions, a mass discriminator and a sensitive detection system (Newman, 1996). The samples are introduced by way of a nebulizer which aspirates the sample with high velocity argon, forming a fine mist. The aerosol then passes into a spray chamber where larger droplets are removed via a drain (Jarvis *et al.*, 1992). Typically, only 2% of the original mist passes through the spray chamber (Olesik, 1996). This process is necessary to produce droplets small enough to be vaporized in the plasma torch. Once the sample passes through the nebulizer and is partially dissolved, the aerosol moves into the torch body and is mixed with more argon gas. A coupling coil is used to transmit radio frequency to the heated argon gas, producing an argon plasma flame located at the torch

(Jarvis *et al.*, 1992). The hot plasma removes any remaining solvent and causes sample atomization followed by ionization. In addition to being ionized, sample atoms are excited in the hot plasma, a phenomenon which is used in ICP-spectroscopy, to enable the ICP-MS to sort isotopes by wave length (since they are ionized particles). The ions are then extracted from the high temperature environment at atmospheric pressure of the plasma into a high vacuum enclosure via an interface region, containing two sequential, millimetre-sized apertures. Focussed by an ion lens system, the isotopes to be analyzed are separated according to their mass/charge ratio by a mass spectrometer (a quadrupole mass spectrometer), and detected and measured by a detector (an electron multiplier).

The following element isotopes were analyzed with the ICP-MS: ^{7}Li, ^{9}Be, ^{11}B, ^{23}Na, ^{24}Mg, ^{27}Al, ^{31}P, ^{39}K, ^{44}Ca, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{88}Sr, ^{105}Pd, ^{107}Ag, ^{116}Sn, ^{118}Sn, ^{137}Ba,^{185}Re, ^{202}Hg and ^{208}Pb.

Sr:Ca, Mn:Ca and Ba:Ca ratios have been used in various studies to detect fish origin (Gao *et al.*, 2005; Muhlfeld *et al.*, 2005; Kalvoda *et al.*, 2009). Ratios with calcium were also calculated on Mg, Al and Fe, due to the fact that they were responsible for much of the differences between groups found in this study. Mn, Mg, Sr and Ba are likely to substitute Ca in the calcified tissue matrix (Patterson and Kingsford, 2005), since their outer electron layer is the same. Neither Al nor Fe substitute Ca in the calcified matrix, but given that Ca levels can be used as a measure for $CaCO_3$ molecule number, the Fe:Ca and Al:Ca ratios can be used as element / $CaCO_3$ ratio. The ratios were calculated with the following

formulae: (Sr x 10)/Ca, (Mn x 10^5)/Ca, (Ba x 10^5)/Ca, (Mg x 10^2)/Ca, (Fe x 10)/Ca and (Al x 10^3)/Ca. Since the ratios are individually compared and not between themselves, different multiplication factors for each ratio were used in order to avoid 10^{-x} values. All the ratios underwent univariate statistical analysis.

Limits of detection were calculated as 3 times the standard deviation of the sample blanks that were run every 10 study samples (Muhlfeld *et al.*, 2005) and standard reference material (SRM) was provided by the ICP-MS technical services of the University of Alicante.

2.7. Statistical analysis

Total fish lengths (TL) underwent univariate analysis to check differences between groups. Trace element concentrations, as well as element /Ca ratios, were checked for correlations with the total fish length.

In order to detect differences between groups using the total pool of different trace elements, a series of multivariate analysis using PRIMER-E® version 5.2.8 for Windows were performed. As a preliminary analysis, an ordinary dominance plot analysis took place to assess variable strength for scales and otoliths of both species (figure 8). One variable was found to be much stronger than the rest, therefore all data underwent a logarithmic transformation ($\log_{x+1}$) and standardization in order give more importance to elements found in lower

concentrations (Quinn and Keough, 2002; Lo-Yat *et al.*, 2005).

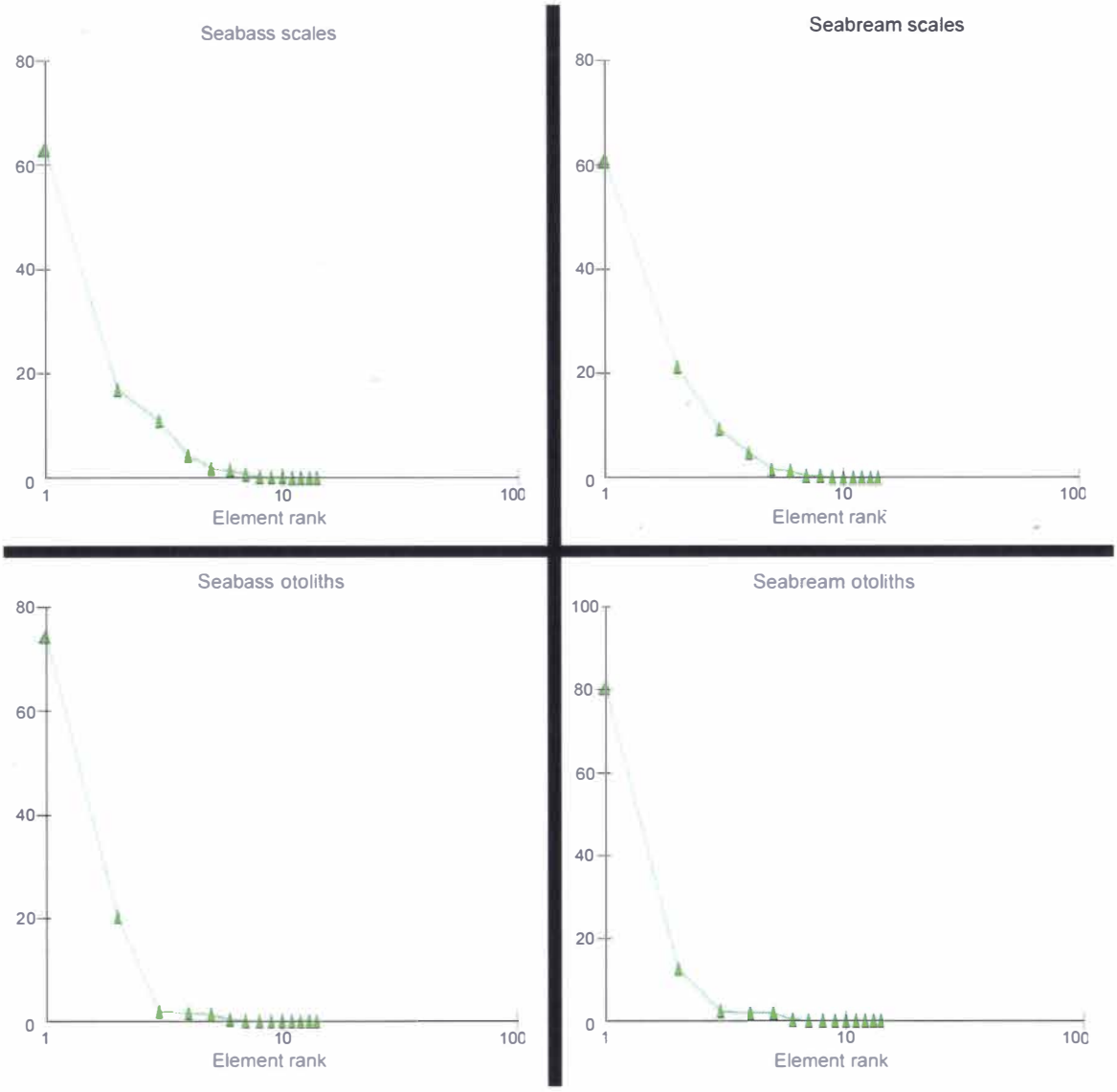

Figure 8. Ordinary dominance plot analysis performed on seabass and seabream scales and otoliths

To obtain triangular similarity matrixes in order to represent in two dimensions the differences using the complete trace element profile of the groups of fish, a Bray-Curtis similarity coefficient was calculated (Clarke and Warwick, 1994) on the data. These triangular matrixes were used to carry out cluster plot dendrograms, non metric multidimensional scaling (nMDS) and analysis of similarities (ANOSIM); all to be described next.

Non transformed, standardized data underwent SIMPER (similarity percentages) analysis in order to detect the similarity degree between and within groups and also the degree of contribution of the elements to the differences and similarities.

2.7.1. Cluster plot dendrogram

In order to have a visual representation of the similarities based on the multi-elemental signatures of the fish tissues, a cluster plot dendrogram was performed on the Bray-Curtis similarity matrix formerly described. The similarity coefficient scale is to be seen in the vertical axis. The cluster plot dendrogram is built by a cumulative and hierarchical ordination of the data based on the Bray-Curtis similarity coefficients of each sample (Clarke and Warwick, 1994).

2.7.2. Multidimensional scaling (MDS)

A non metric multidimensional scaling (nMDS) with 10 restarts was performed on the Bray-Curtis similarity matrix of the $\log_{x+1}$ transformed data described above. Non metric MDS presses the n dimensional space that the analysis creates with the multi-elemental signature of the analyzed tissue into an only two-dimensional space, in order to make graphic display possible. Nevertheless, the displayed distances in the two dimensional representation are equivalent to the actual distances in the n-dimensional space (Clarke and

Warwick, 1994). However, due to this dimensional flattening, some of the dimensions are not distinguishable, and this is indicated by the stress value, shown in the upper right side of the figure (figure 11, page 32). There are no scales shown in the axis of the graph because the distances are only relative to themselves.

2.7.3. Analysis of similarities (ANOSIM)

The ANOSIM performs multiple random permutation methods on the similarity matrix, in order to calculate the R-statistic. This statistic identifies the significant differences that exist in relation to the random distributions that are made from the multiple permutations. The R also indicates the magnitude of the difference between populations and a significant level (Clarke, 1993). The R values range between 0 and 1. An R-statistic value close to 1 ($R > 0.75$) means that there are high differences between the analyzed communities, whereas a value close to 0 ($R < 0.25$) indicates little separation. R values between 0.25 and 0.75 indicate varying degrees of differences between groups. Values lower than 0 may indicate greater similarity between groups than within groups (Chapman and Underwood, 1999). An ANOSIM was performed on the similarity matrix of the data, with 999 maximal permutations to test if differences occurred between farmed and wild fish considering the total trace element signature of the tissue.

2.7.4. SIMPER analysis

SIMPER (similarity percentages) analysis calculates the contribution of each variable to the dissimilarities between groups of samples, as well as the contribution to the similarities within each group. These calculations are based on the Bray-Curtis similarity index. A SIMPER analysis was performed on the non transformed, standardized data matrix, in the present work

2.7.5. Univariate statistical analysis

All univariate analysis have been performed using SPSS® 15.0 for Windows (22nd November, 2006).

All data were tested for normality using Q-Q charts and Kolmogorov-Smirnoff tests for each variable. One-way-ANOVAS were performed for each variable that had a normal distribution. Prior to ANOVA, homocedasticity of the data was checked and data were not transformed, given that ANOVA is robust to heterogeneity of variances (Quinn and Keough, 2002). Post-hoc pairwise comparisons were carried out using Bonferroni and Tukey when the groups were homocedastic and T2 Tamhane and T3 Dunnett when homogeneity of variances was not met. Finally, Student-Newman-Keuls (SNK) post-hoc test was performed when significant differences were found.

3. RESULTS

3.1. Fish lengths

Wild fish total length (TL) showed significant differences with the farmed ones (ANOVA $F = 35.214$, $P < 0.01$ for seabass and $F = 30.126$, $P < 0.01$ for seabream; table II). However, no correlation was found between fish length and any element concentration, so the difference in size is not expected to interfere with the results.

Table II. Total length (mean TL ± standard error, cm) of *Dicentrarchus labrax* and *Sparus aurata* collected from farms A and B and artisanal fisheries (Wild). There are 15 individuals per species and per location (n=90).

Dicentrarchus labrax		***Sparus aurata***	
SITE	**TL**	**SITE**	**TL**
Wild	51.81 ± 1.87	Wild	44.67 ± 1.65
Farm A	41.37 ± 0.14	Farm A	35.59 ± 0.22
Farm B	40.06 ± 0.15	Farm B	35.33 ± 0.18
TOTAL	44.41 ± 1.00	TOTAL	38.53 ± 0.85

3.2. Element selection

Concentrations of ^{9}Be and ^{205}Ti were below the LOD (limit of detection) of the ICP-MS, and were hence discarded from the statistical analysis. The standard reference material was found inaccurate for ^{11}B, ^{23}Na, ^{45}Sc, ^{51}V, ^{105}Pd and $^{116,118}Sn$; those elements were discarded too. Concentrations of ^{7}Li, ^{121}Sb, ^{185}Re and ^{202}Hg were below the LOD in most of the samples. Finally, ^{11}B, ^{23}Na and ^{52}Cr were not detected in 10% or more of the samples. Only elements that were above

the LOD of the ICP-MS in all or most (>90%) of the samples were considered suitable for statistical analysis. Those elements were:

1) For Scales: ^{24}Mg, ^{27}Al, ^{39}K, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{88}Sr, ^{107}Ag, ^{111}Cd, ^{137}Ba and ^{208}Pb
2) For otoliths: : ^{24}Mg, ^{27}Al, ^{31}P, ^{39}K, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{88}Sr, ^{107}Ag, ^{137}Ba and ^{208}Pb

3.3. Scales results

3.3.1. Multivariate analysis

Trace element multi-elemental signature of scales showed significant differences between farmed and wild seabass and seabream. Cluster plot dendrograms indicated that the wild fish were different from the farmed ones at around 92% similarity between groups in both species (figures 9 and 10 respectively). Further, clear differences between farms were found in seabream scale microchemistry. The cluster plot dendrogram revealed differences at a 92% similarity, same as between farmed and wild fish (figure 10).

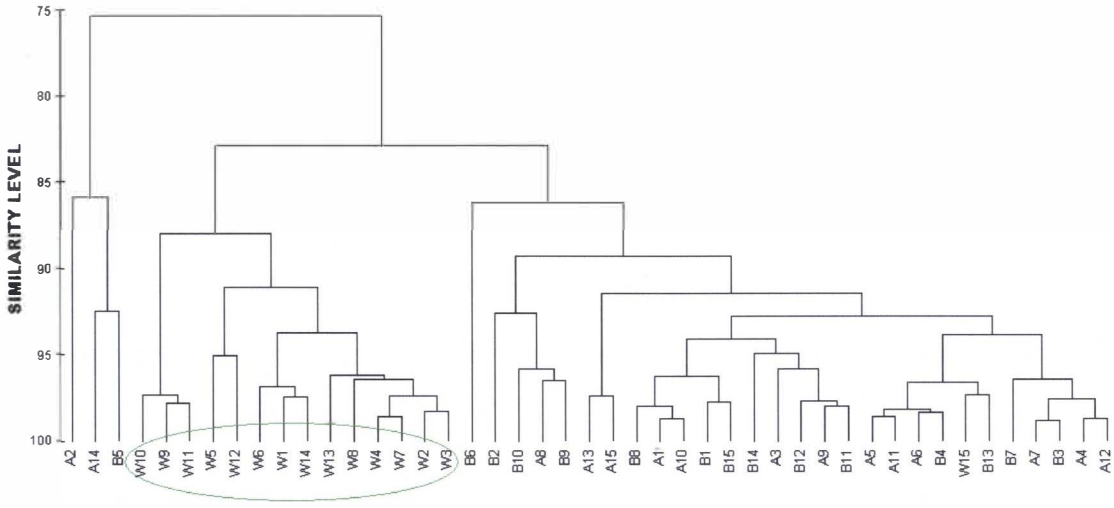

Figure 9. Seabass similarity cluster plot dendrogram. Surrounded in green are the wild fish (W). The group is distinguishable at around 87% similarity level from farms A and B fish.

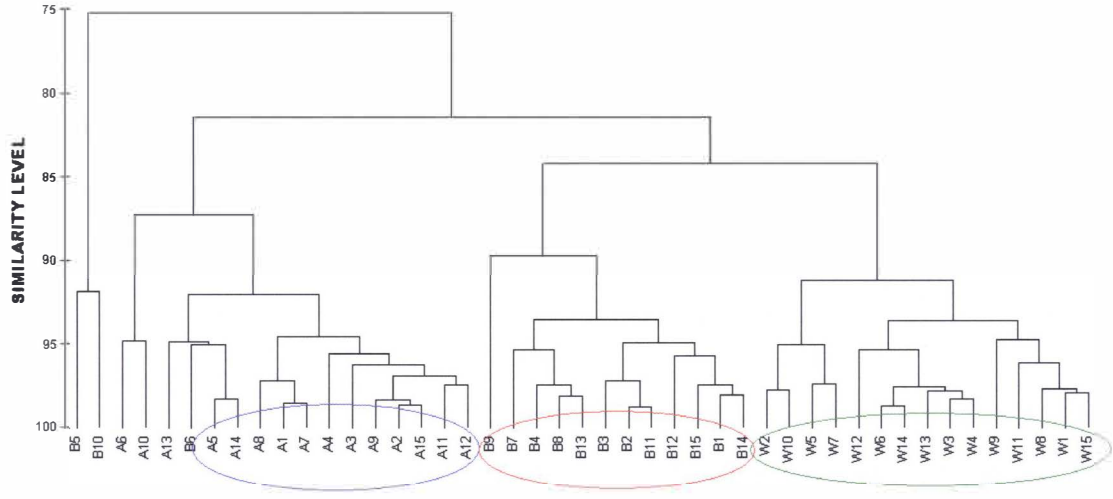

Figure 10. Cluster plot dendrogram of seabream scales. At 92% similarity level there are three different groups for wild (W), farm A and farm B seabream respectively.

Although it may seem in the cluster plot dendrogram that the wild and the farm B seabream are closer to one another than with farm A, SIMPER analysis revealed dissimilarity percentages between wild and each farm that were very similar (18.92% between wild and farm A; 18.78% between wild and farm B). Those

percentages were more than two times higher than the dissimilarity percentage between farms (7.69%). SIMPER performed on seabass scales showed very similar results, being the dissimilarity between wild and farmed fish (11.94% between wild and farm A; 12.07% between wild and farm B) approximately two times higher than the dissimilarity between farms (6.89%), even though these separations between groups were not detected using the cluster plot dendrogram.

The ANOSIM revealed significant dissimilarities between wild and farmed seabass (R = 0.558, Sig. = 0.001 between wild and farm A; R = 0.553, Sig. = 0.001 between wild and farm B), but no significant difference was found between farms (R = 0.002, Sig. = 0.446). Regarding seabream, wild and farmed fish showed an even higher significant dissimilarity (R = 0.955, Sig. = 0.001 between wild and farm A; R = 0.711, Sig. = 0.001 between wild and farm B). Further, significant differences were also found between farms (R = 0.377, Sig. = 0.001). Tables III and IV contain the summarized ANOSIM results.

Table II. ANOSIM performed on seabass scales

	Dicentrarchus labrax	
Pairwise tests	**R**	**Significance**
Wild – Farm A	0.558	0.001
Wild – Farm B	0.553	0.001
Farm A – Farm B	0.002	0.446
Global test	0.355	0.001

Table IV. ANOSIM performed on seabream scales

	Sparus aurata	
Pairwise tests	**R**	**Significance**
Wild – Farm A	0.955	0.001
Wild – Farm B	0.711	0.001
Farm A – Farm B	0.377	0.001
Global test	0.691	0.001

The SIMPER analysis also revealed that the main elements responsible for the differences found between groups in both species were Mg, K, Sr and Al, and they were also contributing the most to the similarities within groups. All these elements were ratio transformed and underwent univariate analysis. However, K was also discarded from the ratios, due to the fact that it is physiologically regulated and hence does not reflect the environment the fish has lived in (Payan *et al.*, 1999). All multivariate analysis was repeated removing K, but the results remained the same.

The spatial representation of these results can be found in figures 11 and 12 using non metric multidimensional scaling plots. Figure 11 shows evidence of one different cluster for wild seabass scale microchemistry. Figure 12 shows three different clusters, one for each farm and one for wild seabream scale multi-elemental signature. All the stresses found in this study were very low (the highest being 0.13 in seabass scales and the lowest 0.06 in seabass otoliths) which means that the distances shown closely match the actual distances in the n-dimensional space.

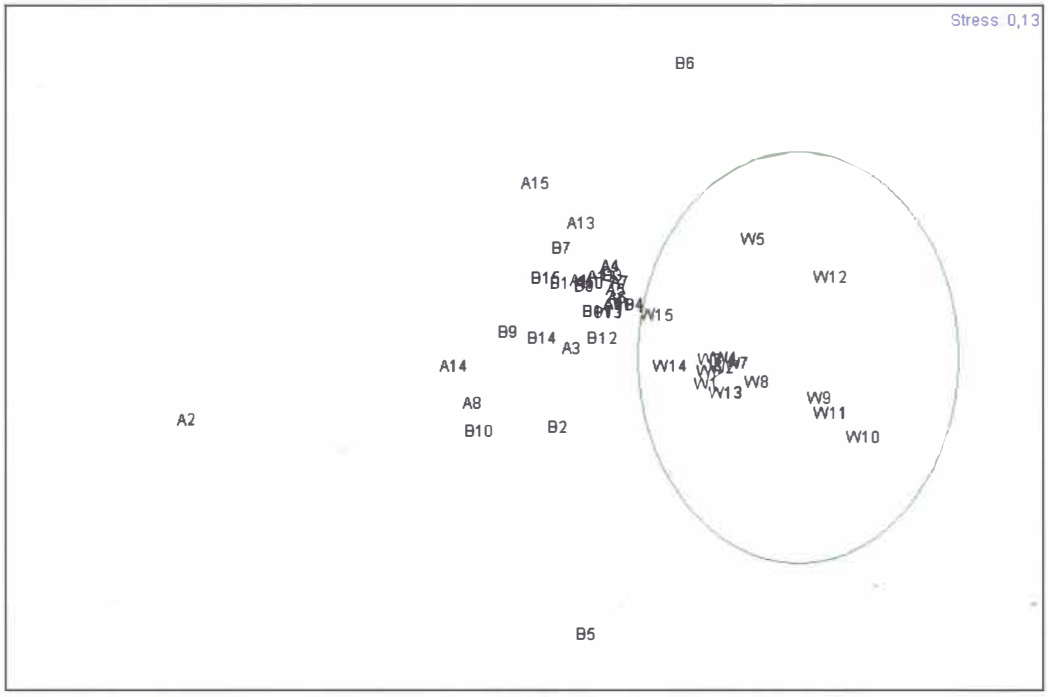

Figure 11. Non metric multidimensional scaling plot of wild (surrounded in green), farm A and farm B seabass based on total trace element compositions of scale samples.

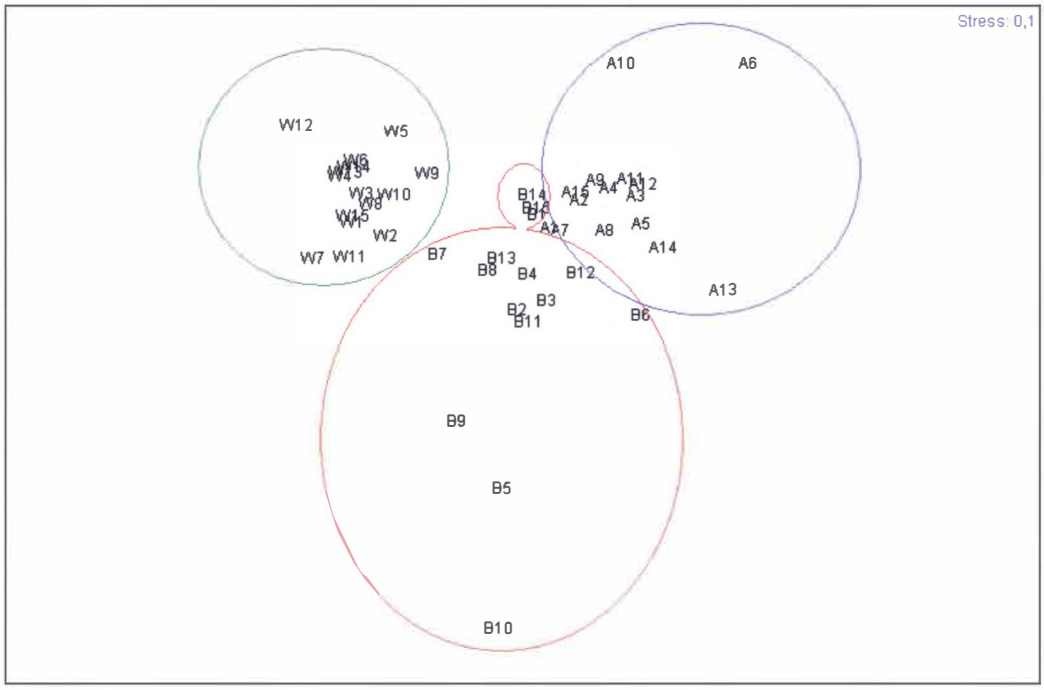

Figure 12. Non metric MDS plot on seabream scales. Wild fish are surrounded in green, farm A in blue and farm B in red.

3.3.2. Univariate analysis

Significant differences between farmed and wild seabass were found regarding Sr:Ca ratio ($p < 0.01$) and Mn:Ca ratio ($p < 0.01$); the highest F being provided by Mn:Ca (table V). All average ratios for seabass are displayed in figure 13, and Mn:Ca was found to be almost 4 times higher in farmed seabass, which is consistent with the fact that it provides the highest F ($F = 84.716$) in the one way ANOVA.

Seabream scale Sr:Ca, Mn:Ca and Mg:Ca ratios also revealed significant differences between farmed and wild fish (all $p < 0.01$). Tamhane pairwise comparisons for each variable showed significant differences between farms regarding Sr:Ca ratio.

Table III. One way ANOVA performed on seabass scales. Significant differences between farmed and wild fish were found regarding Sr:Ca and Mn:Ca ratios.

Ratio	F	Significance
Sr:Ca	24.908	P<0,01
Mn:Ca	84.716	P<0,01
Mg:Ca	1.465	P=0,243
Ba:Ca	1.654	P=0,204
Al:Ca	1.647	P=0.205

Table IVI. One way ANOVA performed on seabream scales. Significant differences were found between groups regarding all ratios except Ba:Ca.

Ratio	F	Significance
Sr:Ca	138.527	P<0.01
Mn:Ca	7.715	P<0.01
Mg:Ca	39.475	P<0.01
Ba:Ca	2.382	P=0.105
Al:Ca	3.436	P=0.041

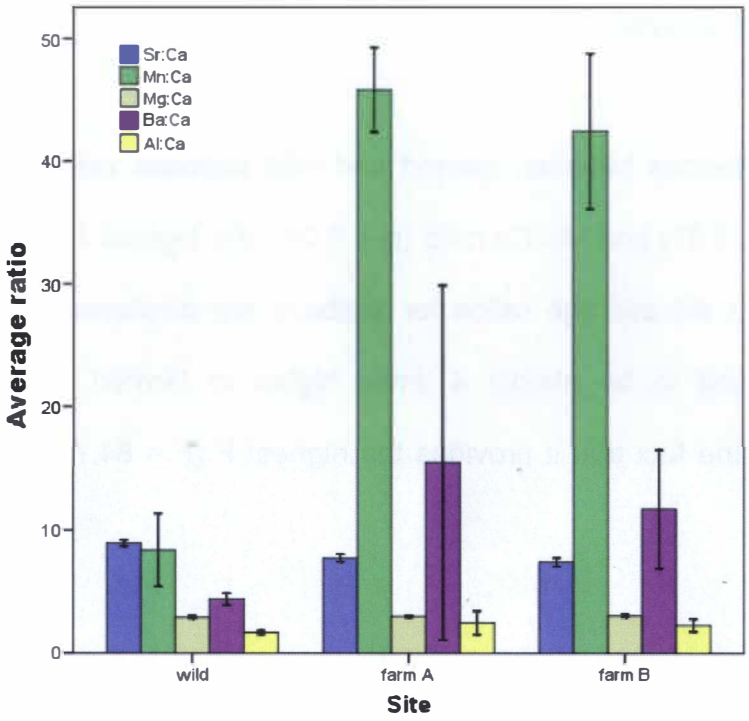

Figure 13. Seabass scale element ratios (mean value ± standard error). Every ratio is only relative to itself and not proportional to the other ratios.

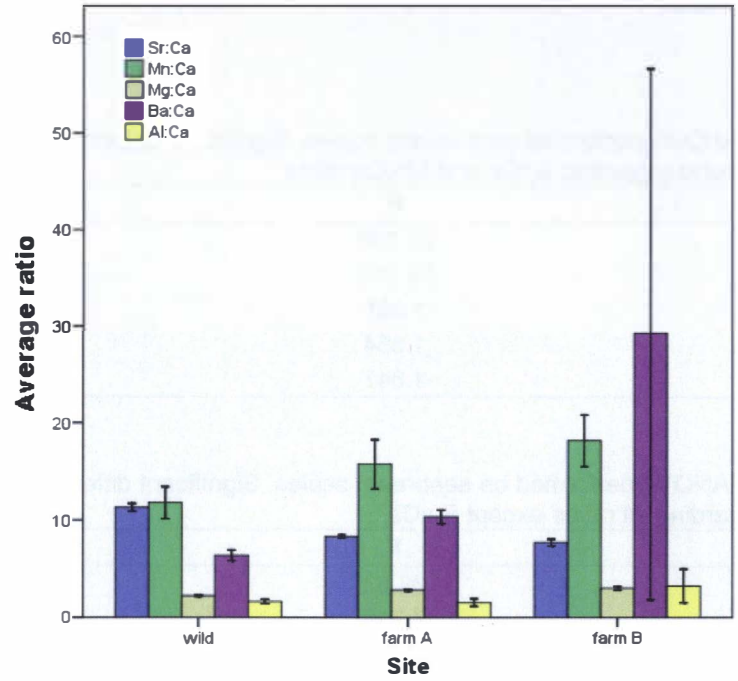

Figure 14. Seabream scales element ratios (mean value ± standard error). Every ratio is only relative to itself and not proportional to the other ratios.

This ratio had also the highest F levels (see table VI); and S-N-K post-hoc test produced different groups for each seabream origin (wild, farm A and farm B). The average ratios can be seen in figure 14.

3.4. Otoliths results

3.4.1. Multivariate analysis

Significant differences were found between wild and farmed fish only regarding seabream otoliths. The cluster plot dendrogram (figure 16) revealed a separate cluster for wild seabream at 86% similarity level. No distinct clustering appeared in seabass otoliths (figure 15) at any similarity level

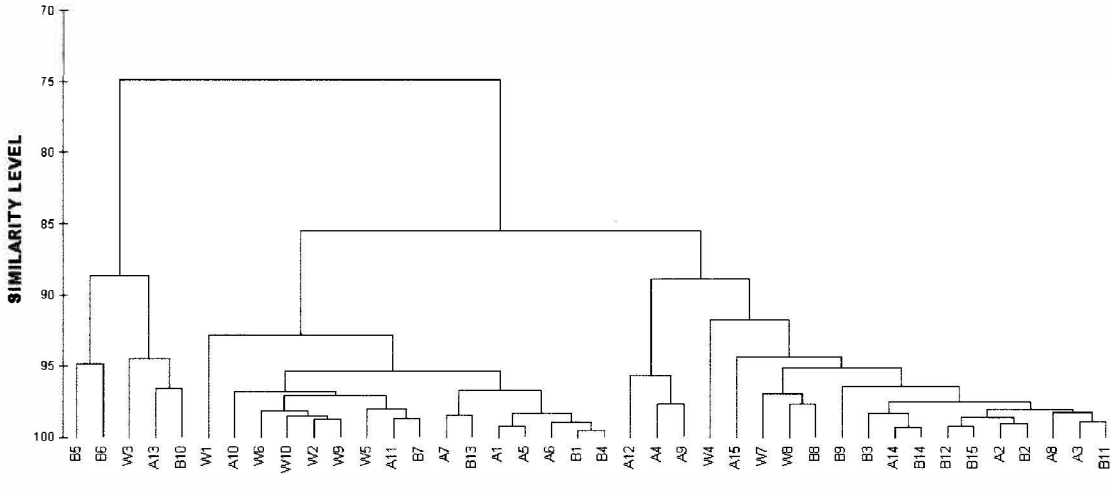

Figure 15. Seabass otoliths cluster plot similarity dendrogram. The groups (A and B for farmed fish, W for wild fish) are not distinguishable at any level of similarity.

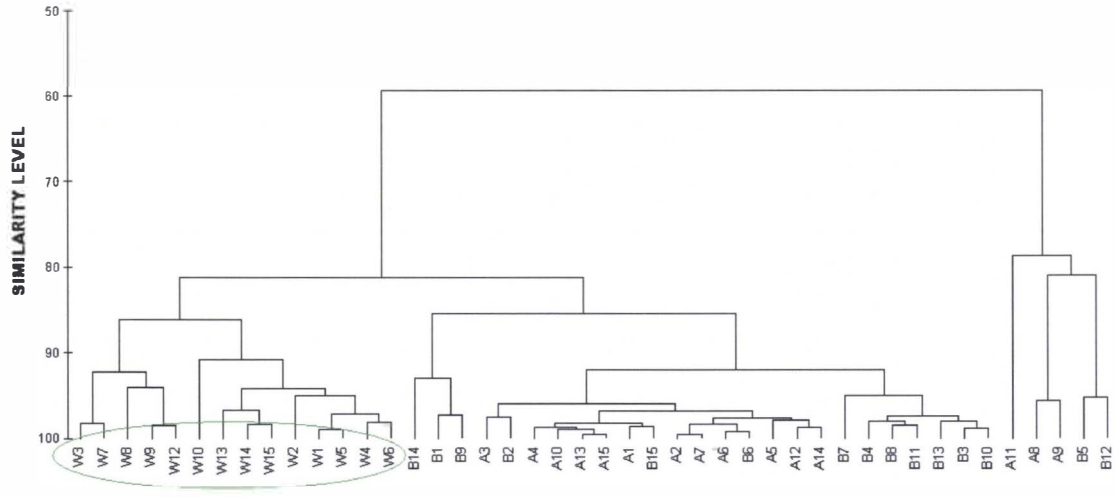

Figure 16. Seabream otoliths similarity cluster plot dendrogram. Surrounded in green are the wild fish (W), distinguishable at around 86% similarity level.

The ANOSIM performed on the similarity matrix revealed significant dissimilarities between wild and farmed seabream (R=0.698, Sig. = 0.001 between wild and farm A; R=0.548, Sig. = 0.001 between wild and farm B). SIMPER analysis detected dissimilarities between wild and farms A and B of 12.67% and 11.27% respectively. However, no such separation arose between farms (R = 0.186, Sig = 0.001). Seabass otoliths revealed no significant dissimilarities among groups whatsoever (see tables VII and VIII).

Table V. ANOSIM performed on seabass otoliths

	Dicentrarchus labrax	
Pairwise tests	**R**	**Significance**
Wild – Farm A	0.198	0.022
Wild – Farm B	0.157	0.033
Farm A – Farm B	-0.032	0.800
Global test	0.088	0.033

Table VI. ANOSIM performed on seabream otoliths

	Sparus aurata	
Pairwise tests	**R**	**Significance**
Wild – Farm A	0.698	0.001
Wild – Farm B	0.548	0.001
Farm A – Farm B	0.186	0.001
Global test	0.440	0.001

SIMPER also showed that the dissimilarities found among groups were mainly due to Sr, Mg, K and Fe in both species. All these elements (except K) were ratio transformed with Ca.

The visual representation of the results using nMDS plots can be found in figures 17 and 18. No different clusterings for any seabass group can be seen.

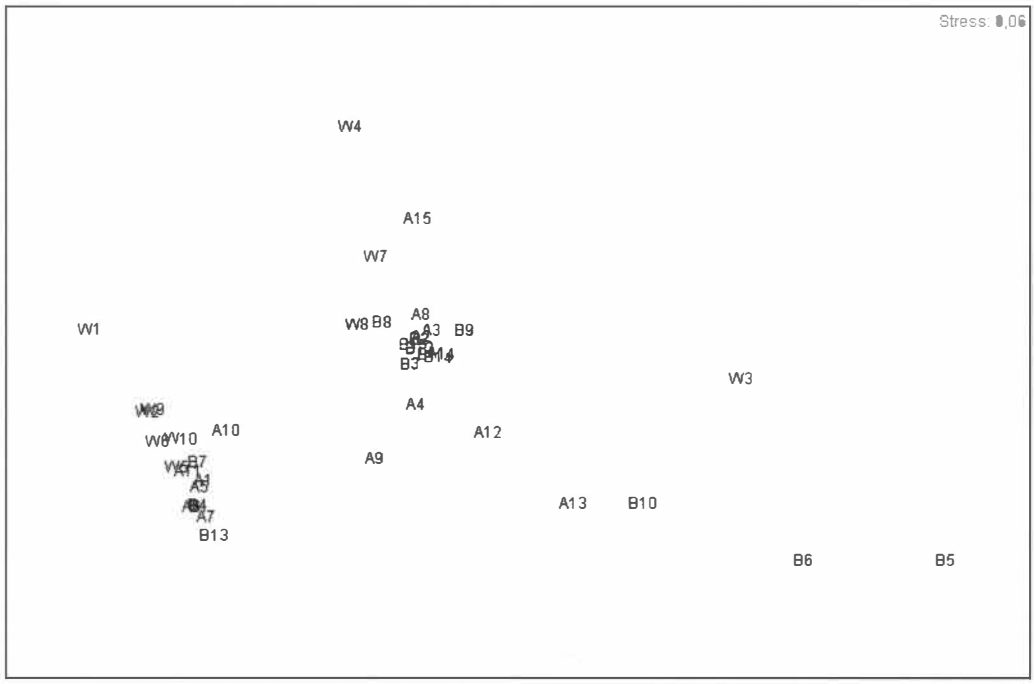

Figure 17. Non metric multidimensional scaling performed on *Dicentrarchus labrax* otoliths. No tendencies can be perceived for farmed (A and B) or wild (W) fish.

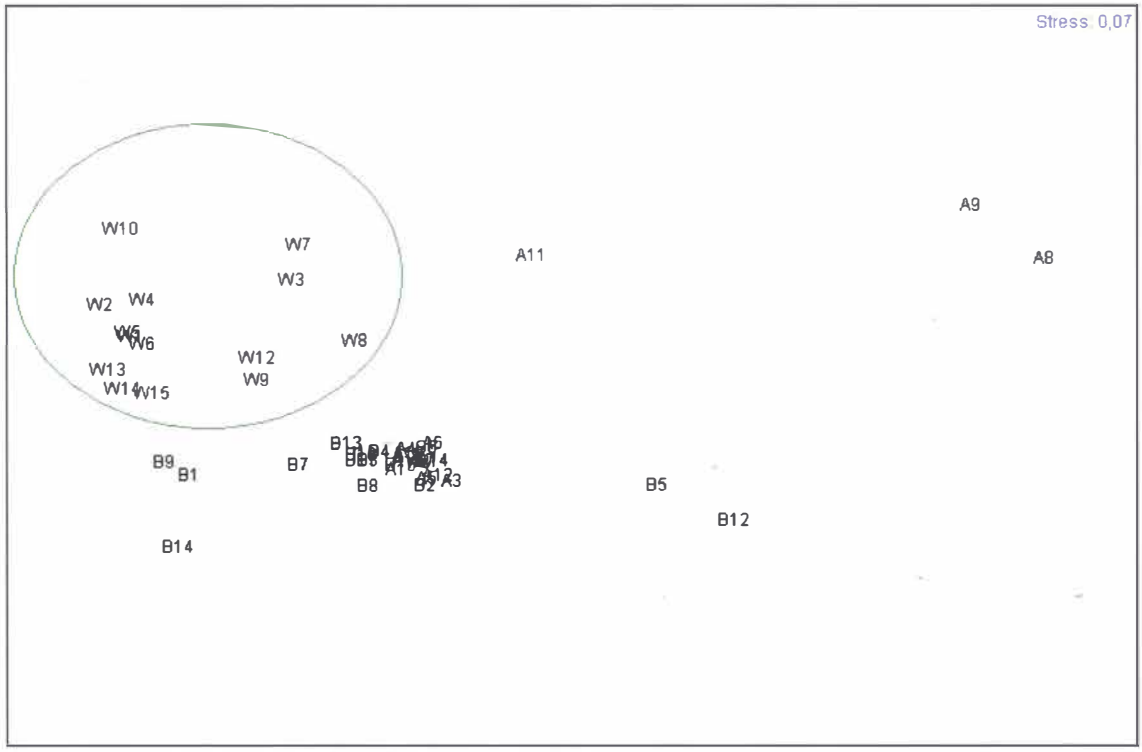

Figure 18. Non metric multidimensional scaling carried out on seabream otoliths. The wild seabream (W) are surrounded in green.

Seabream presented the only trend separating wild from farmed fish. The stresses in both plots are the lowest in this study (0.06 and 0.07), meaning a close two-dimensional representation of the actual distances in the n-dimensional space.

3.4.2. Univariate analysis

Significant differences between farmed and wild fish groups were found on seabass regarding Mn:Ca and Ba:Ca ratios ($p < 0.01$), and on seabream regarding Sr:Ca, Mn:Ca and Fe:Ca ratios. No significant differences were found between farms regarding any ratio in any species. The highest F values were reached by

seabass Mn:Ca ratio (F = 15.949) and seabream Ba:Ca ratio (F = 17.845). See tables IX and X for summarized one way ANOVA outcomes. No significant difference between farms was found using Tukey, Bonferroni and Tamhane pairwise comparisons. Average ratios and standard errors can be seen in figures 19 and 20 for seabass and seabream respectively.

Table IX. Summarized one way ANOVA performed on seabass otoliths. Significant differences were found between groups regarding Mn:Ca and Ba:Ca ratios.

Ratio	F	Significance
Sr:Ca	1.342	P=0.274
Mn:Ca	15.949	P<0.01
Mg:Ca	1.695	P=0.198
Ba:Ca	7.950	P<0.01
Fe:Ca	3.073	P=0.058

Table VII. Summarized one way ANOVA performed on seabream otoliths. Significant differences were found between groups regarding Sr:Ca, Mn:Ca, Ba:Ca and Fe:Ca ratios.

Ratio	F	Significance
Sr:Ca	5.574	P<0.01
Mn:Ca	6.267	P<0.01
Mg:Ca	1.226	P=0.304
Ba:Ca	17.845	P<0.01
Fe:Ca	7.548	P<0.01

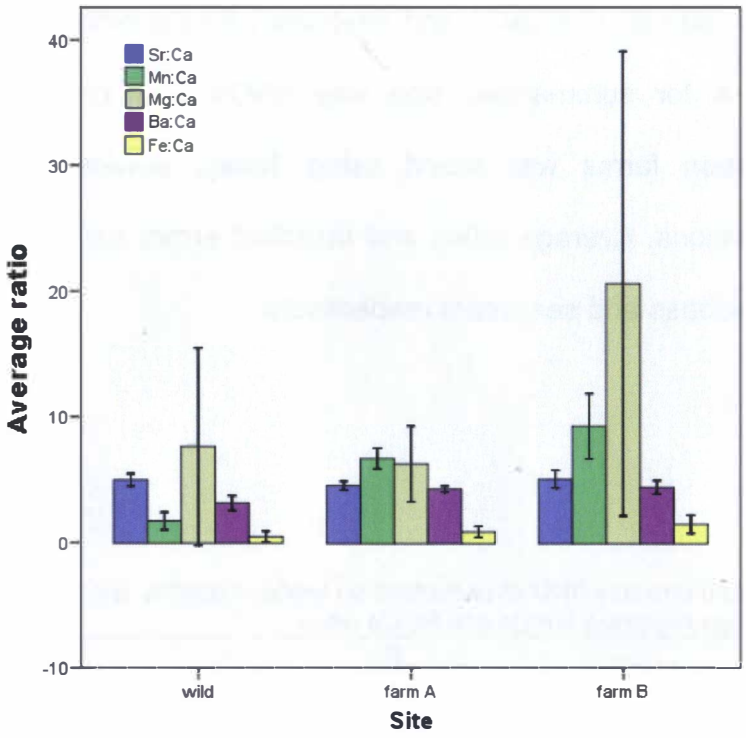

Figure 19. Seabass otolith element ratios (mean value ± standard error). Every ratio is only relative to itself and not proportional to the others.

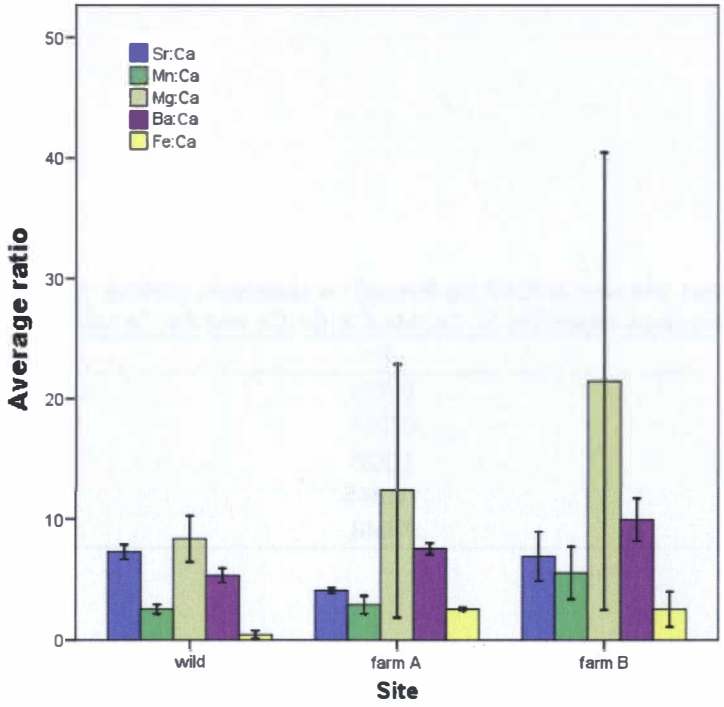

Figure 20. Seabream otolith element ratios (mean value ± standard error). Every ratio is only relative to itself and not proportional to the others.

4. DISCUSSION

Trace element signature in scales showed the sharpest differences between farmed and wild fish for the present study. Multivariate statistics considering total trace element concentrations together, as well as univariate analysis on the selected ratios, were both found useful to tell apart farmed from wild fish. Differences between farms were only found using multivariate analysis and univariate analysis on Sr:Ca ratio on seabream scales.

Regarding otolith trace element signature, differences between farmed and wild fish were also found. Seabream otolith microchemistry revealed significant differences between farmed and wild fish using multivariate analysis, and also when performing univariate analysis on Sr:Ca, Mn:Ca, Ba:Ca and Fe:Ca ratios. Nevertheless multivariate analysis of seabass otoliths did not show differences between groups and the only significant differences between groups analyzing seabass otoliths appeared in Mn:Ca and Ba:Ca ratios. Lack of difference in otolith multi-elemental signature has been also documented for other fish, such as the two banded bream (*Diplodus vulgaris*), in the same coastal region in the south-west Mediterranean (Gillanders *et al.* 2001). Since seabream otoliths positively revealed differences between wild and farmed fish, and knowing that trace element deposition rate is species-specific (Adey, 2007), further research needs to be carried out in order to find proper natural tags for seabass otoliths.

Mn has been previously identified as an important predictor element in determining fish origin (Brophy *et al.*, 2004). This is consistent with the results of this thesis, where Mn:Ca ratio was the only one showing significant differences between farmed and wild fish in both species and both tissues. Furthermore, Mn:Ca ratio was found to be much greater in farmed than in wild seabass. Adey *et al.* 2007 found in Atlantic salmon that differences in Mn:Ca between farmed and wild fish were so large that any variations found between fish from different farms had no effect on the discriminatory ability of Mn. A different study suggested that the Mn:Ca ratio in scales may provide useful information, although there may be post-depositional alterations of trace element composition (Wells *et al.* 2003b).

Sr has the same outer electron layer as Ca, being both adjacent alkaline earth elements in the periodic table and the Sr:Ca ratio is normally higher in blood than in the endolymph (Kalish, 1991), which makes it easier for Sr to substitute Ca in scales than in otoliths. This was confirmed by the present work, where Sr:Ca ratios were around twice higher in scales than in otoliths. Several studies have used Sr:Ca ratio to identify fish origin (Kafemann *et al.*, 2000; Secor and Rooker, 2000; Tsukamoto and Arai, 2000; Campbell *et al.*, 2002) and a study of Elsdon and Gillanders (2006) has confirmed that the otolith Sr:Ca ratio is strongly correlated with the environmental Sr:Ca ratio, masking the influence of other factors on Sr:Ca levels (Elsdon and Gillanders 2003). In the present study, Sr:Ca in scales and otoliths was found useful to distinguish between farmed and wild seabass and seabreams, with the exception of seabass otoliths.

Ba:Ca ratio has been widely used for ecological and paleoecological reconstructions and identification of herbivores, as Ba:Ca ratio decreases as the trophic level grows (Elias *et al.*, 1982; Sillen and Kavanagh, 1982; Sealy and Sillen, 1988; Sillen *et al.*, 1989; Burton and Price, 1990; Katzenberg and Harrison, 1997; Burton *et al.*, 1999; Sponheimer and Lee-Thorp, 2006). The affinity of Ba to vegetal food (Lambert and Weydert-Homeyer, 1993) was in agreement with the slightly higher levels of Ba:Ca ratio found in farmed fish. Seabass and seabream are carnivore species that, under sea cage farming conditions, ingest vegetable oils included in the food pellets (Fernández-Jover *et al.*, 2007). Even so, significant differences regarding Ba:Ca ratios were only found in seabream otoliths. This may be due to the relative short period that farmed fish are fed vegetal matter and to the little amount of it that is included in the food pellets. Nevertheless, due to the high standard deviation of Ba:Ca ratio in both species' scales, it is advisable to repeat the analysis with a larger amount of fish.

The SIMPER analysis exposed that most of the dissimilarities between farmed and wild fish (and between farms for seabream scales) were due to 4 elements. Nevertheless, repeating the multivariate analysis only with these four elements made the separation between groups much blurrier and the stress of the multidimensional scaling grew. The most accurate identification of farmed individuals was achieved when performing multivariate analysis on all 14 elements that contributed to the dissimilarities, according to the SIMPER analysis. Analyzing more or less elements in the ICP-MS does not increase sample preparation complexity or economic cost, hence there is no reason for analyzing fewer

elements.

One aim of the present study was to find a non lethal natural tagging for farmed fish, since otolith collection involves sacrifice of the fish and scales provide a non lethal alternative as natural markers (Coutant and Chen 1993, Gillanders, 2001). Previous studies have found a high correlation between scale and otolith microchemistry (Muhlfeld *et al.*, 2005), which would allow using scales, rather than otoliths, for natural tagging. No such correlation was found in the present work, but in any case, scales were found to be more useful than otoliths to tell apart farmed from wild fish. Several studies favor the use of otolith microchemistry in spite of scales (Campana *et al.*, 1994; Rieman *et al.*, 1994; Thorrold *et al.*, 1998), since they provide a permanent record of the environmental history of the fish, whereas scales are easily replaced and reabsorbed. It still needs to be assessed by further research what is the length of time that scales retain their value as natural markers. Even though their trace element signature is not as stable over time as the otoliths', scales may prove to be a useful mean of identification for short term escapees.

Following the present work, the reliability of the microchemistry of scales and otoliths will be tested using this work's protocol on a more complex study that involves simulating an escape from sea cages. The conditions of an escape from a sea cage will be recreated by releasing artificially tagged farmed fish that will be recaptured later on. This group will be compared to sea cage farmed fish as well as individuals from local fisheries. Artificial tags are necessary because seabream and

seabass occur naturally in the studied environment and it is difficult to accurately identify escaped from wild fish by morphology (Youngson and Hay, 1996) or fatty acid composition (Fernández-Jover *et al.*, 2006).

Aquaculture production has been steadily growing worldwide (FAO, 2007) and escaped individuals pose a threat on the environment whose extent has not been yet completely evaluated. The ability to identify escaped fish, and moreover, to accurately identify the facility from whence the fish escaped, will be one of the basic tools to enhance farm management and to achieve a sustainable aquaculture.

5. CONCLUSIONS

1) Trace element signature of scales reveals more differences between wild and farmed fish than otolith microchemistry.

2) Multi-element signature of seabream scales and otoliths, together with the Sr:Ca and Mn:Ca ratios, are reliable tags for locating the origin of the fish.

3) Mn:Ca ratio shows significant differences between farmed and wild fish in both species and both tissues.

4) Trace element signature has proved to be an efficient natural tag for farmed seabass and seabream in the south western Mediterranean.

6. REFERENCES

ADEY, E. 2007. Distinguishing populations of Atlantic salmon (*Salmo salar*) by elemental analysis of whole scales using Inductively Coupled Plasma Mass Spectrometry. PhD Thesis, University of Aberdeen, United Kingdom. 421 pp

ÅKESSON, S. 2002. Tracking fish movements in the ocean. Trends in Ecological Evolution. 17: 56–57.

ALLEGRUCCI, G., FORTUNATO, C., SBORDONI, V., 1997. Genetic structure and allozyme variation of sea bass (*Dicentrarchus labrax* and *D. punctatus*) in the Mediterranean sea. Marine Biology 128: 347–358.

ARAI, T., GOTO, A., MIYAZAKI, N. 2002. Migratory history of the three spine stickleback *Gasterosteus aculeatus*. Ichthyology research 50: 9 – 14

BAHRI-SFAR, L., LEMAIRE, C., CHATAIN, B., DIVANACH, P., BEN HASSINE, O., BONHOMME, F., 2004. Impact de l'élevage sur la structure génétique des populations Méditerranéennes de *Dicentrarchus labrax*. Aquatic Living Resources 18: 71 - 76

BAUCHOT, M.L. AND J.C. HUREAU 1990 Sparidae. p. 790-812. In J.C. Quero, J.C. Hureau, C. Karrer, A. Post and L. Saldanha (eds.) Check-list of the fishes of the eastern tropical Atlantic (CLOFETA). JNICT, Lisbon; SEI, Paris; and UNESCO, Paris. Vol. 2.

BEACHAM, T.D., WITHLER, R.E. and WOOD, C.C., 1995. Stock identification of sockeye salmon by means of minisatellite DNA variation. North Pacific Anadromous Fish Commission Bulletin, 21: 263 - 276.

BOARDMAN, J., 2008. Facts about Striped Bass. Striperspace.com. Version March 2nd, 2010. <http://www.striperspace.com/facts.html> Downloaded on August 2nd 2010.

BROPHY, D., JEFFRIES, T.E. and DANILOWICZ, B.S. 2004. Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological or structural origins. Marine Biology 144: 779 - 786

BROWN, G.A. and WELLINGS, S.R., 1969. Collagen formation and calcification in teleost scales. Zeitschrift für Zellforschung und mikroskopische Anatomie, 93: 571-582.

BURTON, J.H., PRICE, T.D. 1990. The ratio of barium to strontium as a paleodietary indicator of consumption of marine resources. Journal of Archaeological Science 17: 547 – 557

BURTON, J.H., PRICE, T.D. and MIDDLETON, W.D. 1999. Correlation of bone Ba/Ca and Sr/Ca due to biological purification of calcium. Journal of Archaeological Science 26: 609 – 616

CAMPANA, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine ecology Program series. 188: 263 – 297.

CAMPANA, S.E., 2005. Information for students. Otolith research laboratory. <http://www.marinebiodiversity.ca/otolith/english/home.htm>. Downloaded on August 2nd , 2010

CAMPANA, S.E., FOWLER, A.J. and JONES, C.M., 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using Laser Ablation ICP MS. Canadian Journal of Fisheries and Aquatic Sciences, 51: 1942-1950.

CAMPANA, S.E. and GAGNE, J.A. 1995. Cod stock discrimination using ICP-MS elemental assays of otoliths. Recent developments in fish otolith research. Eds. D.H. Secor, J.M. Dean and S.E. Campana, University of South Carolina Press, Columbia, pp. 671-691.

CAMPANA, S.E. and NEILSON, J.D. 1985. Microstructures of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences, 42: 1014-1032.

CAMPBELL, J.L., BABALUK, J.A., COOPER, M., GRIME, G.W., HALDEN,N.M., NEJEDLY, Z., RAJTA, I., REIST, J.D. 2002 Strontium distribution in young-of-the-year Dolly Varden otoliths: potential for stock discrimination. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 189: 185 – 189

CHAPMAN, M.G. AND UNDERWOOD, A.J. 1999. Ecological patterns in multivariate assemblages: information and interpretation of negative values in ANOSIM tests. Marine Ecology Progress Series ISSN 0171-8630

CLARKE, K.R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117 - 143

CLARKE, M.L., FRIEDLAND, K.D. 2004. Influence of growth and temperature on strontium deposition in the otoliths of Atlantic salmon. Journal of fish biology 65: 744 – 759

CLARKE, K.R. and WARWICK, R.M. 1994. Change in marine communities: an approach to statistical analysis and interpretation. Natural Environmental Research Council, Plymouth, UK. 144 p.

CLAYTON, B., BRISBIN, J.A., SUTTON, K.L. and CARUSO, J.A. 2000. New approaches for elemental speciation using plasma mass spectrometry. American Laboratory. 32(3): 17 - 32.

COUTANT, C.C. and CHEN, C.H. 1993 Strontium microstructure in scales of freshwater and estuarine striped bass *Morone saxatilis* detected by laser ablation mass spectrometry. Canadian Journal of Fisheries and Aquatic Science 50: 1318 – 1323.

CUBILLOS, L.A. 2009. Age verification and growth of the Chilean cardinalfish *Epigonus crassicaudus* (de Buen, 1959) admitting ageing error. Revista de Biología Marina y Oceanografía 44(2): 417-427 <http://www.scielo.cl> Downloaded on August 1st, 2010

DE BRUYN, A.M.H., TRUDEL, M., EYDING, N., HARDING, J., MC NALLY, H., MOUNTAIN, R., ORR, C., VERENITCH, S. and MAZUNDER, A. 2006. Ecosystemic effects of salmon farming increase mercury contamination in wild fish. Environmental science technology. 40: 3489 - 3493

DIAMANT, A., BANET, A., UCKO, M., COLORNI, A., KNIBB, W. and KVITT, H. 2000. Mycobacteriosis in wild rabbitfish *Siganus rivulatus* associated with cage farming in the Gulf of Eilate, Red Sea. Diseases of Aquatic Organisms 39, 211-219.

DIAMANT A., UCKO, M., PAPERNA, I., COLORNI, A., and LIPSHITZ, A. 2005. *Kudoa iwatai* (Myxosporea: Multivalvulidae) in wild and cultured fish in the Red Sea: re-description and molecular phylogeny. Journal of Parasitology 91(5), 1175-1189.

DIMITRIOU, E., KATSELIS, G., MOUTOPOULOS, D.K., AKOVITIOTIS, C., KOUTSIKOPOULOS, C. 2007. Possible influence of reared gilthead sea bream (*Sparus aurata*, L.) on wild stocks in the area of the Messolonghi lagoon (Ionian Sea, Greece). Aquaculture Research 38: 398-408

DOVE, S.G. and KINGSFORD, M.J. 1998. Use of otoliths and eye lenses for measuring trace-metal incorporation in fishes – a biogeographic study. Marine biology 130: 377–387

ELIAS, R.W., HIRAO, Y. and PATTERSON, C.C. 1982. The circumvention of the natural biopurification along nutrient pathways by atmospheric inputs of industrial lead. Geochimica et Cosmochimica Acta 46: 2561–2580

ELSDON, T.S. and GILLANDERS, B.M. 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. Canadian Journal of Fisheries and Aquatic Science 59: 1796 – 1808

ELSDON, T.S. and GILLANDERS, B.M., 2003. Reconstructing migratory patterns of fish based on environmental influences on otolith chemistry. Reviews in Fish Biology and Fisheries 13: 219 – 235

ELSDON, T.S., GILLANDERS, B.M. 2006 Temporal variability in strontium, calcium, barium, and manganese in estuaries: implications for reconstructing environmental histories of fish from chemicals in calcified structures. Estuarine and coastal shelf science 66: 147 – 156

FALINI, G., FERMANI, S., VANZO, S., MILETIĆ, M. and ZAFFINO, G. 2005. Influence on the formation of aragonite or vaterite by otolith macromolecules. European Journal of Inorganic Chemistry 2005: 162 - 167

FAO, 2007. FishStat Plus. Aquaculture production: quantities 1950 – 2005. FAO Rome

FERNÁNDEZ-JOVER, D., LÓPEZ, J.A., SÁNCHEZ-JEREZ, P., BAYLE-SEMPERE, J., GIMÉNEZ, F., MARTÍNEZ, F.J. and DEMPSTER, T., 2007 Changes in body condition and fatty acid composition of wild Mediterranean horse mackerel (*Trachurus mediterraneus* Steindachner 1868) associated to sea cage fish farms. Marine environmental research 63: 1 - 18

FLEM, B., MOEN, V. and GRIMSTVEDT, A. 2005. Trace element analysis of scales from four populations of Norwegian Atlantic salmon (*Salmo salar* L.) for stock identification using laser ablation inductively coupled plasma mass spectrometry. Applied Spectroscopy 59: 245 - 251.

FLEMING, I.A., HINDAR, K., MJØLNERØD, I.B., JONSSON, B., BALSTAD, T. and LAMBERG, A. 2000. Lifetime success and interactions of farmed salmon invading a native population. Proceedings of the Royal Society of London B 267: 1517 - 1523

FRIMODT, C. 1995. Multilingual illustrated guide to the world's commercial warm water fish. Fishing News Books, Osney Mead, Oxford, England. 215 p.

GAO, Y., BARGMANN, G.G., BRAND, U. and NOAKES, D.L.G. 2005. Stable isotopic and trace elemental compositions of otoliths and the stock structure of Pacific cod, *Gadus macrocephalus*. Environmental Biology of Fishes 74: 335 - 348

GAULDIE, R.W., GRAYNORTH, E.J. and ILLINGWORTH, J., 1980. The relationship of the Fe content of some fish otoliths to temperature. Comparative Biochemistry and Physiology, 100A: 19 - 24.

GILLANDERS, B.M., 2001. Trace metals in four structures of fish and their use for estimates of stock structure. Fisheries Bulletin, 99: 410 - 419.

GILLANDERS, B.M., SÁNCHEZ-JEREZ, P., BAYLE-SEMPERE, J. and RAMOS-ESPLÁ, A. 2001. Trace elements in otoliths of the two-banded bream from a coastal region in the south-west Mediterranean: are there differences among locations?. Journal of Fish Biology 59: 350 - 363

GORDON, J.D.M. and SWAN, S.C., 2002. Otolith microchemistry as a means of identifying stocks of deep-water demersal fish (OTOMIC). Report from the Scottish Association for Marine Science, Institut Mediterrani d'Estudis Avançats and Port Erin Marine Laboratory, University of Liverpool. 122 pp.

HANSEN, L.A., DALE, T., UGLEM, I., AAS, K., DAMSGÅRD, B., BJØRN, P.A. 2008. Escape related behaviour of Atlantic cod (*Gadus morhua* L) in a farm situation. Applied Animal Behavioural Science 104: 236 - 250

HANSEN, L.P. and JACOBSEN, J.A. 2003. Origin and migration of wild and escaped farmed Atlantic salmon, *Salmo salar* L., in oceanic areas north of the Faroe Islands. ICES journal of Marine Science 60: 110 – 119

HINDAR, K., FLEMING, I.A., MCGINNITY, P., DISERUD, O. 2006. The genetic and ecological effects of salmon farming on wild salmon: modeling from experimental results. ICES Journal of Marine Science 63: 1234 - 1247

HILLIVIRTA, P., IKONEN, E. and LAPPALAINEN, J., 1998. Comparison of two methods for distinguishing wild from hatchery-reared salmon (*Salmo salar* L.) in the Baltic Sea. ICES Journal of Marine Science 55: 981 - 986.

ICES WGEIM 2006. Risk analysis of the potential interbreeding of wild and escaped farmed seabass and seabream. Report of the working group on environmental interactions of mariculture (WGEIM). Annexes 6 and 7, pp 128 - 155

ISLAM, M.S. 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: Review and analysis towards model development. Marine pollution bulletin 50(1): 48-61

IUCN 2007. Guide for the sustainable development of Mediterranean aquaculture. Interactions between aquaculture and the environment. IUCN, Gland, Switzerland and Málaga, Spain. 107 pp

JARVIS, K.E., GRAY, A.L., and HOUK, R.S. 1992. Handbook of Inductively Coupled Plasma Mass Spectrometry. Chapman and Hall: New York. 380 pp.

KAFEMANN, R., ADLERSTEIN, S., NEUKAMM, R. 2000. Variation in otolith strontium and calcium ratios as an indicator of life history strategies of freshwater fish species within a brackish water system. Fisheries Research 46: 313 – 325

KALISH, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. Journal of Experimental Marine Biology and Ecology 132: 151 – 178

KALISH, J.M. 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non anadromous salmonids. Fish Bulletin 88: 657 – 666

KALISH, J.M. 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbattus*. Marine Ecology Progressive Series 74: 137 – 159

KALVODA, J., NOVÁK, M., BÁBEK, O., BRZOBOHATÝ, R., HOLÁ, M., HOLOUBEK, I., KANICKÝ, V. and ŠKODA, R. 2009. Compositional changes in fish scale hydroxylapatite during early diagenesis; an example from an abandoned meander. Biogeochemistry 94: 197 - 215

KARAKASSIS, I., TSAPAKIS, M., HATZIYANNI, E., PAPADOPOULOU, K.N., PLAITI, W. 2000. Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. ICES Journal of Marine Science 57(5): 1462 - 1471

KARAKASSIS, I., PITTA, P. and KROM, M. 2005. Contribution of fish farming to the nutrient loading of the Mediterranean. Marine Science 69(2): 313 - 321

KATZENBERG, M.A. and HARRISON, R.G. 1997. What's in a bone? Recent advances in archaeological bone chemistry. Journal of archaeological research 5(3): 265 – 293

KOTTELAT, M. and FREYHOF, J. 2007. Handbook of European freshwater fishes. Kottelat publications, Cornol, Switzerland. 646 p.

KRAUS, R.T. and SECOR, D.H. 2004 Incorporation of strontium into otoliths of an estuarine fish. Journal of Experimental Marine Biology and Ecology 302: 85 – 106

KRKOŠEK, M., FORD, J.S., MORTON, A., LELE, S., MYERS, R.A. and LEWIS, M.A. 2007. Declining wild salmon populations in relation to parasites from farmed salmon. Science 318: 1772 - 1775

KRKOŠEK, M., LEWIS, M.A. and VOLPE, J.P. 2005. Transmission dynamics of parasitic sea lice from farm to wild salmon. Proceedings of the Royal Society bulletin 272: 689 - 696

LAMBERT, J.B. and WEYDERT-HOMEYER, J.M. 1993. The fundamental relationship between ancient diet and the inorganic constituents of bone as derived from feeding experiments. Archaeometry 35: 279 – 294

LEMAIRE, C., ALLEGRICCI, G., NACIRI, N., BAHRI-SFAR, L., KARA, H., and BONHOMME, F., 2000. Do discrepancies between microsatellite and allozyme variation reveal differential selection between sea and lagoon in the sea bass (*Dicentrarchus labrax*) ? Molecular Ecology 9: 457 – 467

LLORIS, D. 2002. A world overview of species of interest to fisheries. Chapter: *Dicentrarchus labrax*. Retrieved on 05 May 2005, from www.fao.org/figis/servlet/species?fid=2291. 3p. FIGIS Species Fact Sheets. Species Identification and Data Programme-SIDP, FAO-FIGIS

LLORIS, D. 2005. A world overview of species of interest to fisheries. Chapter: *Sparus aurata*. Retrieved on 08 July 2005, from www.fao.org/figis/servlet/species?fid=2384. 3p. FIGIS Species Fact Sheets. Species Identification and Data Programme-SIDP, FAO-FIGIS.

LO-YAT, A., MEEKAN, M., MUNKSGAARD, N., PARRY, D., PLANES, S., WOLTER, M. AND CARLETON J. 2005. Small-scale spatial variation in the elemental composition of otoliths of *Stegastes nigricans* (Pomacentridae) in French Polynesia. Coral Reefs 24: 646-653

LUND, R.A. and HANSEN, L.P., 1991. Identification of wild and reared Atlantic salmon, *Salmo salar* L., using scale characters. Aquaculture and Fisheries Management, 22: 499 - 508.

MARTIN, G.B., THORROLD, S.R., JONES, C.M. 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). Canadian Journal of Fisheries and Aquatic Science 61: 34 – 42

MCGINNITY, P., PRODÖHL, P., FERGUSON, A., HYNES, R., Ó MAOILÉIDIGH, N., BAKER, N., COTTER, D., O'HEA, B., COOKE, D., ROGAN, G., TAGGART, J. and CROSS, T. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London B 270: 2443 -2450

MORTON, A., ROUTLEDGE, R.D., and WILLIAMS, R. 2005. Temporal patterns of sea lice infestation on wild Pacific salmon in relation to the fallowing of Atlantic salmon farms. North American Journal of Fisheries Management 25(3): 811 - 821.

MUHLFELD, C.C., MAROTZ, B., THORROLD, S.R. and FITZGERALD, L. 2005. Geochemical signatures in scales record stream of origin in westslope cutthroat trout. Transactions of the American Fisheries Society 134: 945 – 959

MURUA, H. AND F. SABORIDO-REY 2003. Female reproductive strategies of marine fish species of the North Atlantic. Journal of Northwest Atlantic Fisheries Science 33:23-31.

MUUS, B.J. AND NIELSEN, J.G. 1999. Sea fish. Scandinavian Fishing Year Book, Hedehusene, Denmark. 340 p.

NAYLOR, R., HINDAR, K., FLEMING, I.A., GOLDBURG, R., WILLIAMS, S., VOLPE, J., WHORISKEY, F., EAGLE, J., KELSO, D. and MANGEL, M. 2005. Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. BioScience 55(5): 427 - 437

NEWMAN, A. 1996. Elements of ICPMS. Analytical Chemistry. 68(Jan 1): 46A - 51A.

OLESIK, J.W. 1996. Fundamental Research in ICP-OES and ICPMS. Analytical Chemistry. 68(Aug 1): 469A - 474A

PATTERSON, H.M. and KINGSFORD, M.J. 2005. Elemental signatures of *Acantochromis polyacanthus* otoliths from the Great Barrier Reef have significant temporal, spatial and between-brood variation. Coral Reefs 24: 360 - 369

PAYAN, P., EDEYER, A., DEPONTUAL, H., BORELLI, G., BOEUF, G. and MAYER-GOSTAN, N. 1999. Chemical composition of saccular endolymph and otolith in fish inner ear: lack of spatial uniformity. American Journal of Physiology-regulatory, Integrative and Comparative Physiology 46: 123 – 131

PENDER, P.J. and GRIFFIN, R.K., 1996. Habitat history of Barramundi Lates calcarifer in a north Australian river system based on barium levels in scales. Transactions of the American Fisheries Society, 125: 679-689

QUINN, G.P. and KEOUGH, M.J. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge. 511 pp.

RADTKE,R.L. and SHEPHERD, B.S. 1991. Current methodological refinements for the acquisition of life history information in fishes: paradigms from panoceanic bill fishes. Comparative Biochemistry Physiology A. 100: 323 – 333

RIEMAN, B.E., MYERS, D.L. and NIELSEN, R.L. 1994. Use of otolith microchemistry to discriminate Oncorhynchus nerka of resident and anadromous ori gin. Canadian Journal of Fisheries and AquaticScience 51: 68 – 77

SÁNCHEZ-JEREZ, P., BAYLE-SEMPERE, J., FERNÁNDEZ-JOVER, D., VALLE, C. and DEMPSTER, T. 2007. Ecological relationships between wild fish populations and Mediterranean aquaculture in floating fish cages. CIESM Workshop Monograph 32: 77 - 80

SÁNCHEZ-LAMADRID, A., 2004. Effectiveness of releasing gilthead sea bream (*Sparus aurata*, L.) for stock enhancement in the bay of Cádiz. Aquaculture, 231: 135 – 148

SATHER, P.J., IKONOMOU, M.G., HAYA, K. 2006. Occurrence of persistent organic pollutants in sediments collected near fish farm sites. Aquaculture 254: 234 - 247

SEALY, J.C., SILLEN, A. 1988. Sr and Sr/Ca in marine and terrestrial food webs in the Southwestern Cape, South Africa. Journal of Archaeolical Science 15: 425 – 438

SECOR, D.H., DEAN, J.M. and LABAN, E.H. 1991. Otolith removal and preparation for microstructural examination: a user's manual. Ed. Belle W. Baruch Institute for Marine Biology and Coastal Research. p. 7 - 10

SECOR, D.H.,ROOKER, J.R. 2000. Is otolith strontium a useful scalar of life cycles in estuarine fishes? Fisheries Research 46: 359 – 371

SEPÚLVEDA, F., MARÍN, S. L., AND CARVAJAL., J. 2004. Metazoan parasites in wild fish and farmed salmon. Aquaculture 235: 89 - 100

SILLEN, A., KAVANAGH, M. 1982. Strontium and paleodietary research: a review. American journal of Physical Anthropology 25: 67 – 90

SILLEN, A., SEALY, J.C., VAN DER MERW, N.J. 1989. Chemistry and paleodietary research: no more easy answers. American Antiquity 54: 504 – 512

SKAALA, Ø., DAHLE, G., JØRSTAD, K.E. AND NÆVDAL, G., 1990. Interactions between natural and farmed fish populations: information from genetic markers. Journal of Fish Biology, 36: 449 - 460

SPONHEIMER, M. and LEE-THORP, M.A. 2006 Enamel diagenesis at South African Australopith sites: implications for paleoecological reconstruction with trace elements. Geochimica et Cosmochimica Acta 70: 1644 – 1654

THORROLD, S.R., JONES, C.M., SWART, P.K. AND TARGETT, T.E. 1998. Accurate classification of nursery areas of juvenile weakfish (*Cynoscion regalis*) based on chemical signatures in otoliths. Marine Ecology Progress Series 173: 253 – 265

THORSTAD, E.B., FLEMING, I.A., MCGINNITY, P., SOTO, D., WENNEVIK, V. and WHORISKEY, F. 2008. Incidence and impacts of escaped farmed Atlantic salmon *Salmo salar* in nature. Report from the Technical Working Group on Escapes of the Salmon Aquaculture Dialogue, January 2008. 108 pp

THRESHER, R.E.1999. Elemental composition of otoliths as a stock delineator in fishes. Fisheries Research, 43: 165 - 204

TOLEDO, K., SÁNCHEZ-JEREZ, P., GONZÁLEZ-LORENZO, G., BRITO, A. 2009. Detecting the degree of establishment of a non-indigenous species in coastal ecosystems: seabass *Dicentrarchus labrax* escapes from sea cages in Canary Islands (Northeastern Central Atlantic). Hydrobiology 623: 203 – 212

TORTONESE, E. 1986 Moronidae. p. 793-796. In P.J.P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen and E. Tortonese (eds.) Fishes of the north-eastern Atlantic and the Mediterranean. UNESCO, Paris. vol. 2.

TSUKAMOTO, K. and ARAI, T. 2001. Facultative catadromy of the eel *Anguilla japonica* between freshwater and seawater habitats. Marine Ecology Progressive Series 220: 265 – 276

TURUJMAN, S.A., WARNER, W.G., WEI, R.R. and ALBERT, R.H., 1997. Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. Journal of AOAC International 80 (3): 622 - 632

TZENG, W.N. 1994. Temperature effects on the incorporation of strontium in otolith of Japanese eel *Anguilla japonica*. Journal of Fish Biology 45: 1055 – 1066

VOLPE, J.P., GLICKMAN, B.W. and ANHOLT, B.R. 2001. Reproduction of aquaculture Atlantic salmon in a controlled stream channel on Vancouver Island, British Columbia. Transactions of the American Fisheries Society, 130: 489 - 494

WELLS, B.K., BATH, G.E., THORROLD, S.R. and JONES, C.M. 2000 Incorporation of strontium, cadmium, and barium in juvenile spot (*Leiostomus xanthurus*) scales reflects water chemistry. Canadian Journal of Fisheries and Aquatic Science 57: 2122 – 2129

WELLS, B.K., RIEMAN, B.E., CLAYTON, J.L. and HORAN, D.L. 2003a. Relationships between water, otolith and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 132: 409 – 424

WELLS, B.K., THORROLD, S.R. and JONES, C.M., 2003b. Stability of elemental signatures in the scales of spawning weakfish, *Cynoscion regalis*. Canadian Journal of Fisheries and Aquatic Sciences, 60: 361 - 369

WILLIAMS, J.P. 2009. Vateritic Otoliths. Jonathan P. Williams Marine Research. http://www.jpwmarineresearch.com/ > Downloaded on August 2nd, 2010

WORTHY, W. 1988. Scope of ICP/MS expands to many fields. Chemical and Engineering News. 66(June 27): 33 - 4

YOUNGSON, A. and HAY, D., 1996. The Lives of Salmon. Swan Hill Press, Shrewsbury, 144 pp.

YOUNGSON, A.F., JORDAN, W.C., VERSPOOR, E., CROSS, T.F. and FERGUSON, A., 2003. Management of salmonid fisheries in the British Isles: towards a practical approach on populations genetics. Fisheries Research, 62: 193 - 209