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Differentiating the wild or farmed origin of Mediterranean fish: a review of tools for sea bream and sea bass

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Abstract

Based on the fact that farmed fish experience different environments, stocking densities and feeding regimes compared with wild fish, several techniques have been developed to discriminate the wild or farmed origin of fish. These techniques quantify differences between genetics, chemical characteristics, fatty acid compositions, trace elements, pollutants, stable isotopes, morphology and organoleptic characteristics. Gilthead sea bream and European sea bass are the most important marine fish in Mediterranean aquaculture and are highly appreciated by commercial and recreational fisheries. A total of 60 studies that used techniques to discriminate farmed from wild fish for sea bream and sea bass form the basis of this review. The most common technique used differences in the lipid and fatty acid composition of fish. Many of these studies dealt with food science and product quality, rather than tracing escapees. A wide range of identification tools is useful in determining the correct origin of captures and proper labelling of marketed fish. External appearance and morphometry are useful for rapid assessments and can be achieved with high accuracy and little cost, especially for sea bream. This makes these methods suitable for detecting large and recent escape events, applicable in fisheries studies, and for ensuring that wild and farmed fish are separated in the marketplace. Techniques using differences in chemical or genetic composition are more useful for environmental monitoring, as they have higher accuracy and can detect escapees long after the escape incident. Regulatory bodies should legislate protocols that describe the technique(s) that must be applied in specific circumstances.

Key words: aquaculture, escape, fish marketing, fisheries, impact, management.

Introduction

The rise of modern aquaculture in the sea has introduced novel impacts into coastal ecosystems. Chief among these for the culture of finfish species in sea-cages is the escape of domesticated individuals from farms and their dispersal into wild fish populations. Once in the wild, escaped individuals disperse from their cages to nearby farm facilities and coastal areas (Uglem *et al.* 2010; Arechavala-Lopez *et al.* 2011, 2012d) which may spread diseases or parasites to other farms or species (Arthington & Blühdorn 1998), and have ecological and genetic consequences for local fish populations (Naylor *et al.* 2005). Gilthead sea bream (*Sparus aurata* Linnaeus 1758) and European sea bass (*Dicentrarchus labrax* Linnaeus 1758) are the most important marine fish in Mediterranean aquaculture. This industry has grown over the past two decades (FAO 2011) as has concern over the escape of domesticated fish into wild populations. More than 200 000 tons per year is now produced in the Mediterranean Sea with an estimated 300–500 million sea bass and sea bream combined held in sea-cages at any one time. Fish escape from farm facilities as a consequence of technical and operational failures (Jensen *et al.* 2010), but the proportions of escapes in the Mediterranean that are due to storms, holes in the netting caused by wear and tear, abrasion on cage ropes, predators or biting by farmed fish within the cage, or spills during handling of fish are difficult to determine (Dempster *et al.* 2007). Indeed, little is known of the scale of sea bream or sea bass escapes from sea-cage cultivation as no Mediterranean country requires mandatory reporting of escape events.

Apart from the negative effects of escapees on the aquaculture industry image, farmed fish can affect wild populations through predation and competition for food, space and breeding opportunities, the spread of parasites and diseases, and interbreeding with wild fish (Jonsson & Jonsson 2006; Grigorakis & Rigos 2011). Escaped sea bream and sea bass are able to survive for months in the wild, swimming away from their escape point to other nearby farm facilities, fishing grounds, coastal habitats and local harbours, where they feed on natural prey and compete for natural resources with wild populations (Toledo-Guedes *et al.* 2009; Arechavala-Lopez *et al.* 2011, 2012d). Farmed sea bream, intentionally released into coastal waters for re-stocking purposes, were found mixed with wild conspecifics showing the same spawning behaviour as wild specimens after one year in the natural environment (Sanchez-Lamadrid 2004). This strongly suggests that gene flows would occur between escaped cultured sea bream and wild populations. Opportunities for interbreeding in sea bass are likely, but have not been reported (Bahri-Sfar *et al.* 2005). Aquaculture and local fisheries also interact when fishers capture farm-aggregated wild fish and farmed escapees. An increase of escapees in fisheries landings has been recorded during recent years in Mediterranean coastal areas, which is accompanied by a decrease both in the mean size and the price of individuals (Dimitriou *et al.* 2007). Occasionally, frauds mislabelling the marketed fish occur, affecting the guarantee of fish quality for the consumer. The temptation to label farmed fish as wild fish by some fish merchants, retailers and restauranteurs may arise because of the price premium commanded by wild fish. However, little effort has been made to develop verifiable and robust methods to distinguish farmed from wild fish to combat mislabelling and to conform to legislation (Bell *et al.* 2007; Morrison *et al.* 2007).

This evidence highlights the importance of developing cost-effective tools to detect farmed individuals within wild stocks. Effective tools will improve knowledge of the frequency and extent of escape events, help to assess potential genetic and ecological risks of escapees to wild populations, and assess the contribution of escapees to fisheries landings. A number of stock identification methods have been developed for fisheries-related applications. Adapting these techniques to distinguish between wild and escaped farmed fish began in the mid-1980s when, for simple and quick identification, a combination of several techniques were used routinely to survey the amount of farmed escapees in catches of salmon (Fiske *et al.* 2005). More recently, several studies on the discrimination of farmed and wild sea bream and sea bass have been published that have compared genetics, chemical characteristics, fatty acid composition, levels of certain trace elements, concentrations of certain pollutants, stable isotope concentrations, morphology and organoleptic characteristics (Table 1). This review summarizes the existing methods applied to sea bream and sea bass to determine differences among farmed and wild individuals, from the easiest and cheapest, to the most labour-intensive or expensive. Further, the applicability and suitability of such techniques were evaluated as tools to differentiate escapees within wild stocks for future sustainable aquaculture and fisheries management.

Literature analysis

Sixty publications were reviewed (58 peer-reviewed articles and two reports) on differences between wild and farmed fish, specifically for sea bream and sea bass (Table 1). A wide variety of approaches and techniques exist in the literature and the specific purpose of studies varies widely among publications. Fatty acids and/or lipid analyses have been used most frequently (63% of total reviewed works; Fig. 1a). Studies based on morphological differences between wild and farmed sea bream and sea bass are also numerous (37%), as well as those that have used the proximate composition of tissues to differentiate wild and farmed fish (27%). Techniques using stable isotopes and pollutants have been used less frequently (12% each).

The reviewed studies were classified according to the aims and scope of each journal (Fig. 1b). Studies relating to food science (i.e. food chemistry, organoleptic studies and culinary interest) were most common, followed by those dealing with aquaculture (aquatic food resources) and pollutants (environmental pollution and monitoring assessment). Studies with a focus on marine biology, ecology, ichthyology and veterinary aspects were less abundant. The main research driver appears to be determining nutritional flesh quality differences (mainly lipids and FAs composition) from the standpoint of both the producer and the consumer, to better inform farmers, retailers and consumers of the effects of the different food production methods on the final product quality.

Analytical tools used to discriminate wild and farmed fish

External appearance and morphology

External appearance and morphological characteristics to some degree reflect the life history of fish (Grigorakis

Table 1 Reviewed works about differentiating farmed and wild fish origin on *Sparus aurata* (A) and *Dicentrarchus labrax* (B)

Authors	GEN	PC	FA/L	SI	POL	TE	EAM	OC	Others	Journals
Alarcón *et al.* (2004)	A	–	–	–	–	–	–	–	–	*Aquaculture*
Alasalvar et al. (2002a)	–	B	B	–	–	B	–	–	B	*Food Chem.*
Alasalvar *et al.* (2002b)	–	A	A	–	–	–	–	A	–	*J. Agric. Food Chem.*
Alasalvar *et al.* (2005)	–	–	–	–	–	–	–	A	–	*J. Agric. Food Chem.*
Allegruzzi *et al.* (1997)	B	–	–	–	–	–	–	–	–	*Marine Biology*
Antunes and Gil (2004)	–	–	–	–	B	–	–	–	–	*Chemosphere*
Arechavala-Lopez *et al.* (2012a)	–	–	–	–	–	–	A, B	–	–	*Hydrobiol.*
Arechavala-Lopez *et al.* (2012b)	–	–	–	–	–	–	A, B	–	–	*J. Fish Biology*
Arechavala-Lopez *et al.* (2012c)	–	–	–	–	–	–	A, B	–	–	*J. Appl. Ichthyol.*
Attouchi and Sadok (2010)	–	A	A	–	–	–	–	–	A	*Food Chem.*
Attouchi and Sadok (2012)	–	A	A	–	–	–	–	–	A	*Food Bioprocess Techol.*
Bell *et al.* (2007)	–	–	B	B	–	–	–	–	–	*J. Agric. Food Chem.*
Blanes *et al.* (2009)	–	–	A	–	A	–	–	–	–	*Arch. Environ. Contam. Toxicol.*
Boglione *et al.* (2001)	–	–	–	–	–	–	A	–	–	*Aquaculture*
Carpene *et al.* (1998)	–	–	A	–	–	A	–	–	A	*Fish Physiol. Biochem.*
Carubelli *et al.* (2007)	–	–	B	–	B	–	–	–	–	*Chemosphere*
Çoban *et al.* (2008)	–	–	–	–	–	–	A	–	–	*Turkish Journal of Zoology*
De Innocentis *et al.* (2005)	A	–	–	–	–	–	–	–	–	*Aquaculture*
Del Coco *et al.* (2009)	–	–	A	–	–	–	–	–	A	*Nutrients*
Eaton (1996)	–	–	–	–	–	–	B			*Fish. Res. Tech. Report*
Erdem *et al.* (2009)	–	–	B	–	–	–	–	–	B	*J. Anim. Vet. Adv.*
Fasolato *et al.* (2010)	–	B	B	B	–	–	B	–	–	*J. Agric. Food Chem.*
Fernandes *et al.* (2007)	–	–	–	–	B	B	B	–	–	*Environ. Res.*
Ferreira *et al.* (2010)	–	–	B	–	B	B	B	–	–	*Ecotoxicol. Environ. Safety*
Flos *et al.* (2002)	–	A	A	–	–	–	A	A	A	*Aquacult. Int.*
Fuentes *et al.* (2010)	–	B	B	–	–	B	B	B	B	*Food Chem.*
Grigorakis (2007)	–	A, B	A, B	–	–	–	A, B	A, B		*Aquaculture*
Grigorakis *et al.* (2002)	–	A	A	–	–	–	A	A	–	*Int. J. Food Sci. Techol.*
Grigorakis *et al.* (2003)	–	A	A	–	–	–	–	A	–	*Aquaculture*
Karaiskou *et al.* (2009)	A	–	–	–	–	–	–	–	–	*J. Fish Biology*
Krajnović-Ozretić *et al.* (1994)	–	–	B	–	–	–	B	–	–	*Comp. Biochem. Physiol.*
Lenas *et al.* (2010)	–	–	A, B	–	–	–	–	–	A, B	*Int. Aquat. Res.*
Lo Turco *et al.* (2007)	–	–	B	–	B	–	–	–	–	*Environ. Monit. Assess.*
Loukavitis *et al.* (2012)	A	–	–	–	–	–	–	–	–	*Aquacult. Res.*
Mannina *et al.* (2008)	–	–	B	–	–	–	–	–	B	*Talanta*
Miggiano *et al.* (2005)	A	–	–	–	–	–	–	–	–	*Aquacult. Int.*
Minganti *et al.* (2010)	–	–	–	–	–	A	–	–	–	*Mar. Pollut. Bull.*
Mnari *et al.* (2007)	–	–	A	–	–	–	–	–	–	*Food Chem.*
Mnari *et al.* (2010a)	–	–	B	–	–	B	–	–	–	*African J. Food Sci.*
Mnari *et al.* (2010b)	–	–	A	–	–	–	–	–	A	*Int. J. Food Sci. Techol.*
Mnari *et al.* (2010c)	–	–	A	–	–	–	–	–	A	*J. Agric. Food Chem.*
Monti *et al.* (2005)	–	–	B	–	–	B	–	–	B	*Anal. Chem.*
Moreno-Rojas *et al.* (2007)	–	–	–	A	–	–	–	–	–	*Rapid Commun. Mass Spectrom.*
Morrison *et al.* (2007)	–	–	A	A	–	–	–	–	A	*Lipids*
Nasopoulou *et al.* (2007)	–	–	A, B	–	–	–	–	–	–	*Food Chem.*
Orban *et al.* (2002)	–	B	B	–	–	B	–	–	–	*J. Food. Sci.*
Orban *et al.* (2003)	–	A, B	A, B	–	–	–	–	–	A, B	*J. Food. Sci.*
Ottavian *et al.* (2012)	–	B	B	B	–	–	B	–	–	*J. Agric. Food Chem.*
Palma *et al.* (2001)	A	–	–	–	–	–	A	–	–	*J. Mar. Biol. Ass. UK*
Patarnello *et al.* (1993)	B	–	–	–	–	–	B	–	-	*Mol. Mar. Biol. Biotechnol.*
Periago *et al.* (2005)	–	B	B	–	–	–	B	B	B	*Aquaculture*
Rezzi *et al.* (2007)	–	–	A	–	–	–	–	–	A	*J. Agric. Food Chem.*
Rogdakis *et al.* (2011)	–	–	–	–	–	–	A	–	–	*Int. J. Fish. Aquacult.*
Sağlik *et al.* (2003)	-	–	A, B	-	–	-	–	-	–	*Eur. J. Lipid Sci. Technol.*
Santaella *et al.* (2007)	-	B	B	–	-	B	–	-	B	*An. Vet. (Murcia)*
Serrano *et al.* (2007)	–	–	A	A	–	–	–	–	–	*Chemosphere*

Table 1 (continued)

Authors	GEN	PC	FA/L	SI	POL	TE	EAM	OC	Others	Journals
Serrano *et al.* (2008)	–	–	–	A	A	–	–	–	–	*Sci. Tot. Environment*
Šimat *et al.* (2012)	–	A	–	–	–	–	A	A	A	*J. Appl. Ichthyol.*
Sola *et al.* (1998)	B	–	–	–	–	–	B	–	–	*CIHEAM Opt. Medit.*
Yildiz *et al.* (2008)	–	–	A, B	–	–	–	–	–	–	*Int. J. Food Sci. Techol.*

G, genetic; PC, proximate composition; FA/L, fatty acids/lipids; SI, stable isotopes; POL, pollutants; TE, trace elements; EAM, external appearance and morphology; OC, organoleptic characteristics; Others, i.e. muscle cellularity, muscle activity, free aminoacids, collagen, pH, water activity, water holding capacity, nonprotein nitrogen, etc.

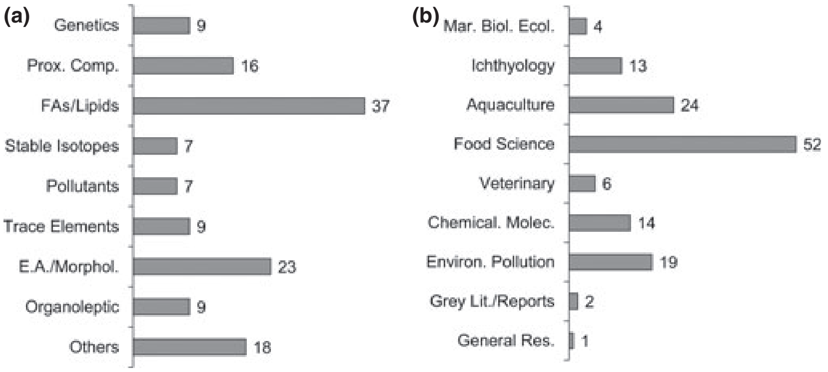


Figure 1 Number of classified works within the literature reviewed: (a) according to each subject within the present review; and (b) according to the aims and scope of the journal.

2007). External characteristics in farmed fish are affected by culture conditions, such as stocking density and feeding strategy (Grigorakis 2007). Several studies document that the morphology of wild sea bream differs significantly from farmed individuals. Wild sea bream have a lower body height, sharper snout and a more squat and compact shape; they are shorter, wider and higher (Flos *et al.* 2002; Grigorakis *et al.* 2002; Rogdakis *et al.* 2011). Farmed sea bream have small, rounded and less developed teeth than their wild counterparts which have bigger, sharper teeth (Grigorakis *et al.* 2002). Skin characteristics can also differ, but differences have not been detected universally. Farmed sea bream have been determined to have thicker skin, which is darker in the dorsal and head areas, and the characteristic iridescent colours are much duller (Grigorakis *et al.* 2002; Rogdakis *et al.* 2011; Šimat *et al.* 2012). In contrast, colour similarities between wild and semi-intensively produced sea bream were found by Flos *et al.* (2002), suggesting that skin pigmentation is related to access to natural rather than commercial food.

Farmed sea bream have fewer scales than wild fish and these are more difficult to detach (Grigorakis *et al.* 2002), with slightly disoriented patterns on scales of the lateral line (Carrillo *et al.* 2001) and regenerated nuclei due to the loss of scales in farming conditions (Katselis *et al.* 2003; Arechavala-Lopez *et al.* 2012b). Differences in the condition of farmed and wild sea bream fins are typically strong. Farmed fish have smaller anal and sharper dorsal fins (Grigorakis *et al.* 2002) and a higher degree of erosion in the caudal and pectoral fins (Arechavala-Lopez *et al.* 2012c). Scale characteristics and fin condition are both strongly related to the stocking density and freedom of movement of farmed sea bream. However, fin erosion values of sea bream escapees were intermediate between those of wild and farmed fish, indicating that fin recovery must be considered when determining fish origin (Arechavala-Lopez *et al.* 2012c).

For sea bass, Eaton (1996) suggested that external differences among wild and cultured fish are not pronounced, and identification cannot rely on shape, colour or general appearance. Fin condition is also difficult to use as an indicator of fish origin in sea bass (Arechavala-Lopez *et al.* 2012c). However, genetic deformities in a number of fin rays and vertebrae on farmed sea bass have been shown (Sola *et al.* 1998). Moreover, farmed sea bass scales, which are fed continuously, have a continuous growth pattern compared with their wild counterparts, which have clear annual rings that indicate large seasonal differences in growth rates (Arechavala-Lopez *et al.* 2012b). Although external characteristics of scales have shown a high accuracy discriminating between wild and farmed sea bream and sea bass (98% and 99% respectively; Arechavala-Lopez *et al.* 2012b), there is no evidence on how the scales of escapees can be modified or regenerated over time.

Significant morphometric differences in cranial and body features, and skeletal abnormalities, exist among fish of farmed or wild origin for sea bream (from larvae to adult) and sea bass (juvenile and adult) (Boglione *et al.* 2001; Rogdakis *et al.* 2011; Arechavala-Lopez *et al.* 2012a). These differences diminished in released sea bream after 6–7 months in the wild, however, mean values and variances of key morphological features remained sufficiently different to distinguish the two groups (Rogdakis *et al.* 2011). However, no differences were found in geometrical morphometry between lagoon caught and cultured fish by Çoban *et al.* (2008), which was thought to be related to similarities in feeding and stocking conditions between culture and lagoon environments.

The condition index is an important tool to measure body shape and acts as a good indicator of dietary condition and history (Grigorakis 2007). Higher condition indices for farmed fish compared with wild fish are observed typically for both sea bream (Flos *et al.* 2002; Rogdakis *et al.* 2011; Arechavala-Lopez *et al.* 2012a) and sea bass (Krajnović-Ozretić *et al.* 1994; Fernandes *et al.* 2007; Fasolato *et al.* 2010; Arechavala-Lopez *et al.* 2012a). The relative profile index and cephalic index have also been suggested as indicators of fish origin for sea bream and sea bass, respectively (Flos *et al.* 2002; Arechavala-Lopez *et al.* 2012a). Other morphometric techniques applied to otoliths have revealed significant differences in otolith shape between wild and farmed fish for sea bream and sea bass (Arechavala-Lopez *et al.* 2012b). Thus, wild and cultured sea bream and sea bass show a range of distinct morphometric differences, which not only indicate dietary condition and history (Grigorakis 2007), but might also be easy, cheap and reliable tools to discriminate fish origin, being suitable for detecting large and recent escapees (see Table 2 for a summary).

Organoleptic characteristics

Organoleptic properties and nutritional value are two sets of characteristics that, together with freshness (quality of appearance, taste and texture), consumers use to determine the quality of fish (Grigorakis 2007). These characteristics strongly depend on the chemical composition of the fish, which in turn depends on the intrinsic characteristics of the fish (e.g. species, age, sex), environmental variables (e.g. temperature, salinity) and feeding history (e.g. diet composition, feeding rate; Grigorakis 1999). The nutritional value and organoleptic characteristics of fish are especially affected by rearing conditions, so that composition and sensory parameters between wild and farmed fish differ (Børrensen 1992). Artificial diets in farmed fish provide a wide range of nutrients, which not only determine fish growth rate but also flesh composition, in particular the lipid content, which may be quantitatively and qualitatively modified. Studies revealing the existence of differences in the organoleptic characteristics between farmed and wild

Table 2 Summary of sea bream and sea bass characteristics according to the wild or farm origin, based on the external appearance, morphology and organoleptic characteristics found in the literature reviewed

Characteristics	*Sparus aurata*		*Dicentrarchus labrax*	
	Farmed	Wild	Farmed	Wild
Body/Shape	Higher body height	Lower body height	Higher K	Lower K
	Higher condition index (K)	Lower K and RPI	Lower cephalic index	Higher cephalic index
	Higher relative profile index (RPI)	Squat and compact shape	Skeletal abnormalities	Slight sharp head
		Sharper snout		
Teeth	Small and less developed	More developed		
	Rounded and squared shape	Sharper and conical		
Skin	Duller colours, darker in head and dorsal areas	Brighter iridescent colours	Shiny silver	High contrast grey colours
	Harder skin with thicker walls	Thinner skin		
Scales	Regenerated nucleus	Clear nucleus	Lack of annual deposition	Clear annual rings
	Fewer density	Higher density		
	Slight disoriented			
Fins	High erosion degree in caudal and pectoral fins	Low erosion signs	Deformities in fin rays	Low probability of abnormalities
	Smaller belly and sharper dorsal fin			
Organoleptic	Whiter appearance	Darker appearance	Higher smell	Fresher smell
	Heavier smell	Softer smell	Higher juiciness	Higher springiness, hardness, cohesiveness, chewiness, gumminess, firmness and deformability
	Higher juiciness	More pleasant taste	Higher tenderness	
	Higher tenderness	Firmer texture		
		Dry/fibrous sensation		

sea bream (Alasalvar *et al.* 2002a, 2005; Flos *et al.* 2002; Grigorakis *et al.* 2002, 2003; Šimat *et al.* 2012) and sea bass (Periago *et al.* 2005; Fuentes *et al.* 2010) are common (Table 2).

Grigorakis *et al.* (2003) found that wild sea bream muscle has a darker appearance compared with the whiter appearance of farmed sea bream. Wild fish have to move continuously, and have higher proportions of dark muscle used for continuous swimming. In contrast, white muscles are used for rapid burst swimming and the higher fat content in cultured fish muscle may contribute to its whiter appearance (Grigorakis *et al.* 2003). The most common sensory descriptors assigned to wild sea bream were a more pleasant taste and a firmer texture than farmed fish (Grigorakis *et al.* 2003).

Fat content strongly affects the impression of taste in the mouth, and differences in the texture of fish muscle have been related to lipid, protein and moisture contents (Venugopal & Shahidi 1996). Fat-rich tissues usually taste very smooth and succulent (‘juicy’), while the sensation of ‘dryness’ or ‘fibrousness’ (‘rough’ or ‘coarse’) describes the tissue better when fat levels are low (Grigorakis *et al.* 2003). The profile of volatile aroma compounds of wild fish differs from those of cultured fish, as they contain a higher number of taste-contributing compounds (Grigorakis *et al.* 2003; Alasalvar *et al.* 2005). Grigorakis *et al.* (2002) defined the external smell of wild sea bream as much softer than farmed sea bream, which sometimes smelt like fish oil. However, sensorial indicators do not consistently provide a clear basis to separate farmed and wild sea bream. A sensorial evaluation by Flos *et al.* (2002) found differences among sea bream from three different inland culture systems but no differences between them and wild conspecifics. In addition, Alasalvar *et al.* (2002a) found that the texture of cultured and wild sea bream stored in ice decreased throughout the storage period, and they were not significantly different until after day 16 when the wild sea bream was significantly softer than the cultured fish. Šimat *et al.* (2012) applied the quality index method, which is a sensory evaluation method using a scoring system, to find differences between wild and farmed sea bream.

For sea bass, significant differences between cultured and farmed fish have been detected for all textural parameters measured. Springiness, hardness, cohesiveness, chewiness, gumminess, firmness and deformability are all .higher in wild compared with farmed specimens (Periago *et al.* 2005; Fuentes *et al.* 2010). The flesh of wild fish is firmer, which could be attributed to its lower fat content and the higher level of swimming activity. High fat content in farmed fish could lead to a poorer texture, but texture is also related to other factors, such as the collagen content of the flesh, pH and the muscle fibre size (Johnston *et al.* 2000; Periago *et al.* 2005). Moreover, farmed sea bass were darker than wild fish, which could be attributed to the differences found in the moisture content (Fuentes *et al.* 2010). Farmed and wild fish differ in proximate composition, colour, texture, fatty acids and free amino acids profiles. Therefore, organoleptic differences can, to a large extent, be related to compositional differences. Thus, the application of a multi-indicator based approach using physico-chemical parameters and organoleptic characteristics could be highly useful to distinguish farmed and wild fish.

Proximate composition and fatty acid profile

In intensive marine aquaculture, the high lipid content of the diet and the intensive feeding regime affect the chemical composition of the fish, resulting in a higher fat content (Lopparelli *et al.* 2004). The vast majority of studies dealing with the muscle proximate composition of sea bream have found that wild individuals have lower lipid and higher water contents than farmed sea bream, both from inland and offshore culture (Carpene *et al.* 1998; Alasalvar *et al.* 2002a; Flos *et al.* 2002; Grigorakis *et al.* 2002, 2003; Orban *et al.* 2003; Nasopoulou *et al.* 2007; Attouchi & Sadok 2010, 2012; Šimat *et al.* 2012) (Table 3). These two parameters are usually inversely correlated and such proportions are attributed to the high dietary fat level in the feed and the reduced activity of cultured fish (Alasalvar *et al.* 2002b;. Whether protein levels differ among farmed and wild fish is more uncertain; some studies have documented higher protein concentrations in wild sea bream muscle compared with farmed fish muscle (Carpene *et al.* 1998; Grigorakis *et al.* 2002; Orban *et al.* 2003), while others have found no significant differences (Alasalvar *et al.* 2002a; Flos *et al.* 2002; Grigorakis *et al.* 2003; Nasopoulou *et al.* 2007; Attouchi & Sadok 2010, 2012). Protein content may not only be determined by diet, but also by species, genetic characteristics and fish size (Grigorakis 2007; Attouchi & Sadok 2010). Furthermore, all previous authors reported no significant differences in ash analysis between sea bream of different origin.

Similar results have been found for sea bass (Table 4); farmed individuals have higher lipid levels than their wild conspecifics, while no clear differences exist for proteins, moisture and ash composition (Alasalvar *et al.* 2002b; Orban *et al.* 2003; Periago *et al.* 2005; Nasopoulou *et al.* 2007; Santaella *et al.* 2007; Fasolato *et al.* 2010; Fuentes *et al.* 2010; Ottavian *et al.* 2012). Therefore, proximal analysis is not a useful technique to determine fish origin, but it provides a basis for further analyses such as metabolic profiling or determining the fatty acid (FA) signature.

Fish lipids are rich in long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), especially eicosapentaenoic acid (EPA: 20:5n-3) and docosahexaenoic acid (DHA: 22:6n-3), which play a vital role in human nutrition, disease preven-

Table 3 Resulting proximate composition and fatty acids profile on different tissues for sea bream *Sparus aurata* from the literature reviewed

Tissue	Proximate composition (%)				Fatty acids profile (%)									Authors
	Lipids	Prots.	Moist.	Ash	18:1n-9	18:2n-6	18:3n-3	22:6n-3	20:5n-3	20:4n-6	n3/n6	MUFA	PUFA	
M	F>W	NS	W>F	NS	–	–	–	–	–	–	–	–	–	Alasalvar *et al.* (2002b)
M	F>W	NS	W>F	NS	–	–	–	–	–	–	–	–	–	Attouchi and Sadok (2010, 2012)
M, L, G	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Blanes *et al.* (2009)
Mw	–	NS	–	–	W>F	F>W	–	F>W	–	–	–	–	–	Carpene *et al.* (1998)
Mr	–	W>F	–	–	W>F	F>W	–	F>W	–	–	–	–	–	Carpene *et al.*(1998)
M	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Del Coco *et al.* (2009)
M	F>W	–	–	–	F>W	F>W	NS	F>W	F>W	W>F	W>F	F>W	F>W	Fernandez-Jover (u.d.)
L	F>W	–	–	–	F>W	F>W	F>W	F>W	F>W	W>F	W>F	F>W	F>W	Fernandez-Jover (u.d.)
M	F>W	NS	W>F	NS	–	–	–	–	–	–	–	–	–	Flos *et al.* (2002)†
M	F>W	W>F	NS	NS	F>W	F>W	F>W	NS	NS	W>F	NS	–	–	Grigorakis *et al.* (2002)
M	F>W	NS	W>F	NS	–	–	–	–	–	–	–	–	–	Grigorakis *et al.* (2003)
B	F>W	–	–	–	F>W	F>W	W>F	F>W	F>W	NS	W>F	W>F	F>W	Lenas *et al.* (2010)
M	F>W	–	–	–	W>F	F>W	F>W	F>W	W>F	W>F	F>W	NS	NS	Mnari *et al.* (2007)
L	F>W	–	–	–	W>F	NS	F>W	F>W	F>W	W>F	F>W	W>F	F>W	Mnari *et al.* (2007)
M	F>W	–	W>F	–	NS	F>W	F>W	F>W	F>W	W>F	F>W	W>F	F>W	Mnari *et al.* (2010b)
M	F>W	–	–	–	W>F	F>W	–	F>W	F>W	W>F	–	–	–	Morrison *et al.* (2007)
M	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Nasopoulou *et al.* (2007)
M	F>W	W>F	W>F	NS	W>F	F>W	F>W	W>F	W>F	W>F	NS	F>W	NS	Orban *et al.* (2003)
M	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Rezzi *et al.* (2007)
M	F>W	–	–	–	F>W	F>W	F>W	F>W	F>W	NS	NS	F>W	F>W	Saglik *et al.* (2003)
S	F>W	–	–	–	F>W	F>W	F>W	NS	NS	NS	W>F	F>W	F>W	Saglik *et al.* (2003)
M, L, G	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Serrano *et al.* (2007)
M	F>W	NS	W>F	NS	–	–	–	–	–	–	–	–	–	Šimat *et al.* (2012)
M	F>W	–	–	–	sv	sv	sv	sv	sv	sv	–	sv	sv	Yildiz *et al.* (2008)

M, muscle; L, liver; G, gills; w, white; r, red; S, skin; B, brain; F, farmed fish; W, wild fish; sv, seasonal variations; NS, no significant differences; u.d., unpublished data.
†Inland culture.

Table 4 Resulting proximate composition and fatty acids profile on different tissues for sea bass *Dicentrarchus labrax* from the literature reviewed

Tissue	Proximate composition (%)				Fatty acids profile (%)									Authors
	Lipids	Prots.	Moist.	Ash	18:1n-9	18:2n-6	18:3n-3	22:6n-3	20:5n-3	20:4n-6	n3/n6	MUFA	PUFA	
M	F>W	NS	W>F	NS	F>W	F>W	NS	W>F	W>F	W>F	W>F	F>W	W>F	Alasalvar *et al.* (2002a)
M	F>W	–	–	–	NS	F>W	–	W>F	W>F	W>F	–	–	–	Bell *et al.* (2007)
M	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Carubelli *et al.* (2007)
M	F>W	–	–	–	F>W	F>W	NS	W>F	F>W	NS	W>F	F>W	W>F	Erdem *et al.* (2007)
M	F>W	F>W	W>F	F>W	F>W	F>W	F>W	W>F	W>F	W>F	W>F	F>W	NS	Fasolato *et al.* (2010)
M	F>W	–	–	–	F>W	F>W	F>W	F>W	F>W	F>W	W>F	F>W	F>W	Fernandez-Jover (u.d.)
L	F>W	–	–	–	F>W	W>F	F>W	F>W	F>W	NS	W>F	NS	NS	Fernandez-Jover (u.d.)
L	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Ferreira *et al.* (2010)
M	F>W	–	–	–	–	–	–	W>F	W>F	–	W>F	F>W	W>F	Ferreira *et al.* (2010)
M	F>W	W>F	W>F	NS	F>W	F>W	NS	W>F	W>F	W>F	W>F	NS	NS	Fuentes *et al.* (2010)
M	F>W	–	–	–	F>W	F>W	W>F	W>F	W>F	–	–	–	W>F	Krajnovic-Ozreti *et al.* (1994)
L	F>W	–	–	–	F>W	F>W	W>F	W>F	W>F	–	–	–	W>F	Krajnovic-Ozreti *et al.* (1994)
B	F>W	–	–	–	F>W	F>W	F>W	F>W	NS	W>F	W>F	W>F	F>W	Lenas *et al.* (2010)
M	NS	–	–	–	–	–	–	–	–	–	–	–	–	Lo Turco *et al.* (2007)
L	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Lo Turco *et al.* (2007)
M	F>W	–	–	–	–	–	–	W>F	W>F	–	–	W>F	W>F	Mannina *et al.* (2008)
S	F>W	–	–	–	–	–	–	W>F	W>F	–	–	W>F	W>F	Mannina *et al.* (2008)
Md	F>W	–	–	–	NS	F>W	NS	NS	NS	W>F	NS	F>W	NS	Mnari *et al.* (2010a)
Mv	F>W	–	–	–	NS	F>W	NS	NS	F>W	W>F	NS	NS	NS	Mnari *et al.* (2010a)
L	F>W	–	–	–	NS	F>W	NS	NS	F>W	W>F	NS	NS	NS	Mnari *et al.* (2010a)
M	F>W	–	–	–	W>F	W>F	W>F	W>F	W>F	F>W	–	–	W>F	Monti *et al.* (2005)
M	F>W	–	–	–	–	–	–	–	–	–	–	–	–	Nasopoulou *et al.* (2007)
M	F>W	NS	W>F	W>F	F>W	F>W	F>W	NS	NS	W>F	NS	W>F	NS	Orban *et al.* (2002)
M	F>W	NS	W>F	NS	F>W	F>W	F>W	NS	NS	W>F	NS	W>F	F>W	Orban *et al.* (2003)
M	F>W	F>W	W>F	F>W	F>W	F>W	NS	W>F	W>F	W>F	W>F	F>W	W>F	Ottavian *et al.* (2012)
M	NS	NS	NS	–	NS	W>F	–	W>F	F>W	–	F>W	F>W	W>F	Periago *et al.* (2005)
M	F>W	–	–	–	F>W	F>W	F>W	F>W	F>W	NS	W>F	F>W	F>W	Saglik *et al.* (2003)
S	F>W	–	–	–	F>W	F>W	F>W	F>W	F>W	NS	W>F	F>W	F>W	Saglik *et al.* (2003)
M	F>W	NS	NS	NS	W>F	F>W	–	W>F	F>W	–	F>W	–	–	Santaella *et al.* (2007)
M	F>W	–	–	–	sv	sv	sv	sv	F>W	NS	–	sv	sv	Yildiz *et al.* (2008)

M, muscle; L, liver; d, dorsal; v, ventral; S, skin; B, brain; F, farmed fish; W, wild fish; sv, seasonal variations; NS, no significant differences; u.d., unpublished data.

tion and health promotion (Horrocks & Yeo 1999). Since the incorporation and storage of FAs in fish tissues strongly depends on the FA profile of the diet (see Sargent *et al.* 2002 for a review), the current practice of substituting fish oils with other vegetable lipid sources in farmed marine fish diets leads to notable changes in lipid composition and FA profiles, due to the presence of FAs of terrestrial origin such as oleic acid (OA: 18:1n-9), α-linolenic acid (LNA: 18:3n-3) or linoleic acid (LA: 18:2n-6), which are usually found at low levels in marine fish. Therefore, differences between wild and farmed dietary FA profiles have been used widely to discriminate farmed and wild fish origin. Several authors have studied the differences in FA composition of different tissues, such as muscle, liver, skin or brain, in both sea bream and sea bass (see Tables 3,4). Generally, the majority of these studies show pronounced differences between wild and farmed fish with respect to LA and ARA, which are found in higher and lower levels, respectively, for both cultured adult sea bass and sea bream (Fig. 2).

However, different patterns have been established for other FAs (including EPA and DHA) and FA indexes between wild and farmed fish because of the high variability and high standard deviations within the reviewed results. Such high heterogeneity is strongly affected by the dietary history and exhibits strong seasonality and spatiality (Grigorakis 2007; Yildiz *et al.* 2008). All farmed fish are not fed with the same diets and there is a high variability of the FA composition of different aqua-feeds available in the market, since LC n-3 PUFA is likely depending on the fish oil used as the lipid source in their diet (Turchini *et al.* 2010). Similarly, there is a big variability in the FA profile from wild fish depending on the availability of food, season and the site at which the fish is caught. Hence, in addition to different levels of LA and ARA, some authors have suggested that the presence in different tissues of specific FAs that come from diets derived from different marine environments to that of the culture site may serve as biomarkers for wild or farmed sea bream and sea bass (e.g. cetoleic acid 22:1n11; Grigorakis *et al.* 2002).

While the presence of characteristic terrestrial fatty acids in farm diets provides strong individual markers, the use of multivariate analysis using FA profiles further strengthens this technique. Multivariate analysis using FA profiles has proved effective in assessing the influence of coastal aquaculture on wild fish stocks (Fernandez-Jover *et al.* 2007, 2009, 2011; Arechavala-Lopez *et al.* 2010). These studies have usually applied gas chromatography, although nuclear magnetic resonance (NMR; Rezzi *et al.* 2007; Mannina *et al.* 2008; Del Coco *et al.* 2009) and near-infrared spectroscopy (NIRS; Ottavian analyses *et al.* 2012) have detected differences between wild and farmed sea bream and sea bass, as well as their geographical origin using lipids, FAs and different metabolites. However, these latter techniques are not used widely for seafood authentication due to problems in procedure standardization (Forshed *et al.* 2003; Rezzi *et al.* 2007; Martinez *et al.* 2009). Differences in lipid extraction, fatty acid esterification and laboratory equipment characteristics create variability among results from different laboratories.

The most important source of concern regarding all these techniques is the 'wash-out' of fatty acids and other metabolites, due to feeding of escaped fish on natural diets once in the wild (Arechavala-Lopez *et al.* 2012d). This process will diminish the 'signature' farm-diet FAs of terrestrial origin over time with the FA profile of the escapee gradually becoming more similar to those of wild fish. The FA signature may reveal clearly the origin of an individual if it has recently escaped from a fish farm after being fed with commercial pellets for a period of time (Fernandez-Jover unpubl. data, 2012). However, post-escape, the possibility exists that the metabolic and FA profiles will gradually modify if a natural diet is sustained over time, presumably weeks to months (Bell *et al.* 2003). How fast a change in fatty acid pattern would occur is not possible to establish with certainty (Olsen & Skilbrei 2010). It is suggested that an escapee captured after months feeding exclusively on natural marine prey will have around 4% 18:2n-6 or less in muscle. However, escaped fish can either consume feed pellets around farms or be starving, and the proportion of many fatty acids remains in similar proportions throughout this period (Johansson & Kiessling 1991; Olsen & Skilbrei 2010). Thus, where detection of long-term escapees is required, other techniques are more appropriate.

Stable isotopes

Stable isotope analysis is a powerful tool in the analysis of trophic relationships in aquatic environments. Carbon (13C), nitrogen (15N) and oxygen (18O) are three of the principal elements that living organisms are composed of and 'propagate' from one organism to another through food webs. Assimilation and growth enriches the isotopic signatures in marine vertebrates (Morrison *et al.* 2007; Serrano *et al.* 2007). The natural diet of wild fish depends on the availability and accessibility of prey in a particular habitat, which differs significantly from a fish farm diet. Farmed fish are cultured on manufactured diets containing lower levels of marine-derived raw materials which are often obtained from non-local marine sources. Farmed fish diets contain a significant terrestrial carbon input from vegetable meals and oils, which may reflect a lighter isotopic content. Based on these differences, stable isotope analysis has been applied to discriminate wild and farmed sea bream and sea bass.

Clear differences exist in the isotopic ratios between wild and farmed fish in different tissues of sea bream (Moreno-

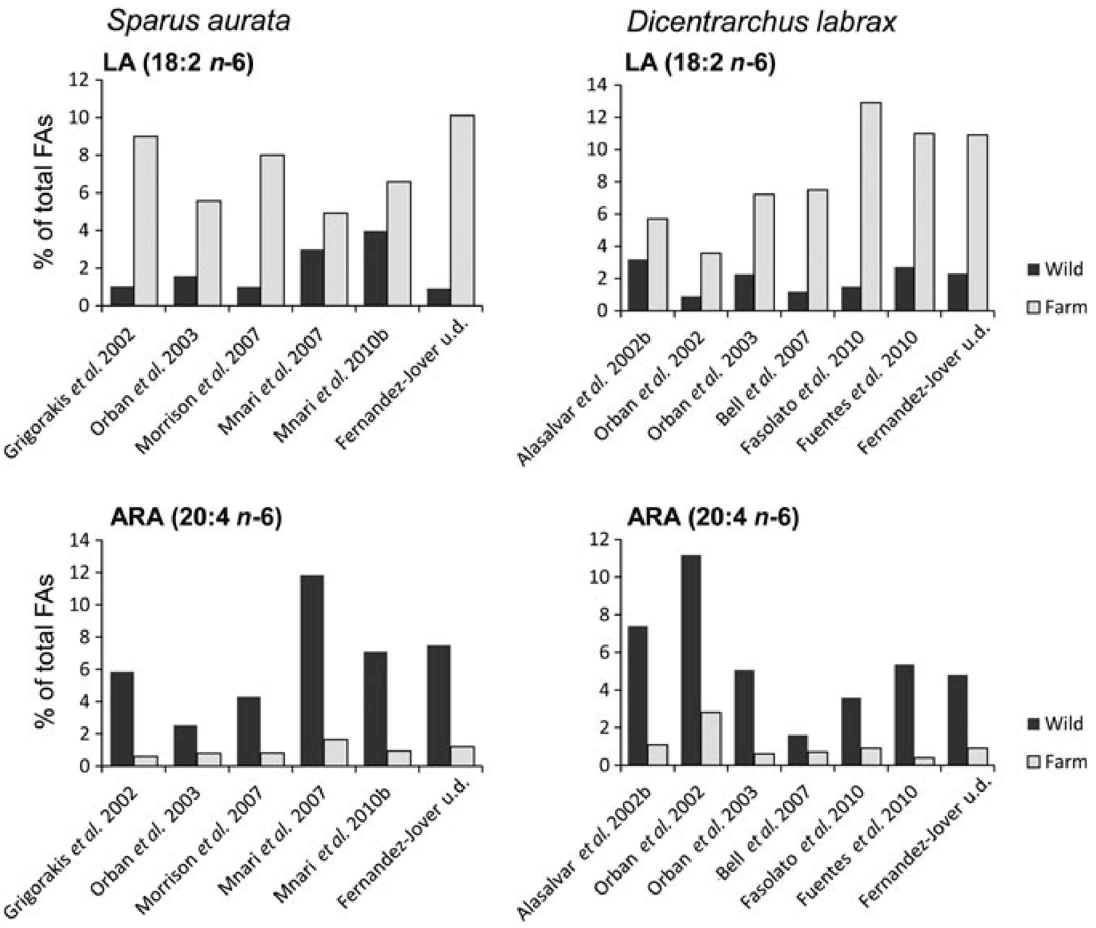


Figure 2 Literature review of linoleic acid (LA) and arachidonic acid (ARA) on sea bream and sea bass fish muscle with significant differences. Percentages of total FAs of wild fish (black bars) and farmed fish (grey bars).

Rojas *et al.* 2007; Morrison *et al.* 2007; Serrano *et al.* 2007, 2008) and sea bass (Bell *et al.* 2007; Fasolato *et al.* 2010; Ottavian *et al.* 2012). δ13C was consistently lighter in farmed fish than wild fish (Fig. 3), which reflects the different diets, and more specifically, the use of terrestrial material (with a lower 13C content) in the feed of farmed fish. Farmed fish have a higher lipid content in the muscle, due to the high-fat diet that produces tissues with a higher lipid content. This induces a larger isotopic fractionation of 13C than that found for wild fish, for which the scarcity of food induces a higher metabolic turnover and results in less accumulation of fat in the tissues. However, different tissues provide variable results. Serrano *et al.* (2007) found δ13C was only depleted in farmed fish tissues with high lipid contents, such as red muscle, the liver and the gills. The variability in tissue lipid content can alter bulk tissue δ13C values (Focken & Becker 1998) and could be falsely interpreted as dietary or habitat shifts (Fasolato *et al.* 2010).

With regard to δ15N, some authors observed significant differences in the nitrogen content and δ15N in muscle tissue between farmed and wild sea bream (Morrison *et al.* 2007) and sea bass (Bell *et al.* 2007; Fasolato *et al.* 2010), where wild fish were isotopically enriched in 15N compared with their farmed counterparts. Such differences can be a consequence of the higher trophic level of the fish feed in the natural marine system, but it also may vary according to the manufactured farmed fish diet characteristics reflecting different inputs from terrestrial N sources in farmed sea bass (Fogel & Cifuentes 1993). Moreno-Rojas *et al.* (2007) suggested that differences in δ15N seemed to be more informative of the geographical origin of fish, which could be related more to differences in feed mixtures given to farmed fish in each area or country than to geological or environmental differences among areas. Moreover, other factors may influence δ15N, such as maturity or growth rate, seasonal variations and analysis techniques (Serrano *et al.* 2007, 2008). The lipid extraction process should not affect the 15N content of the defatted tissue, unless the extraction process leaks proteins linked to lipids (Soritopoulos *et al.* 2004).

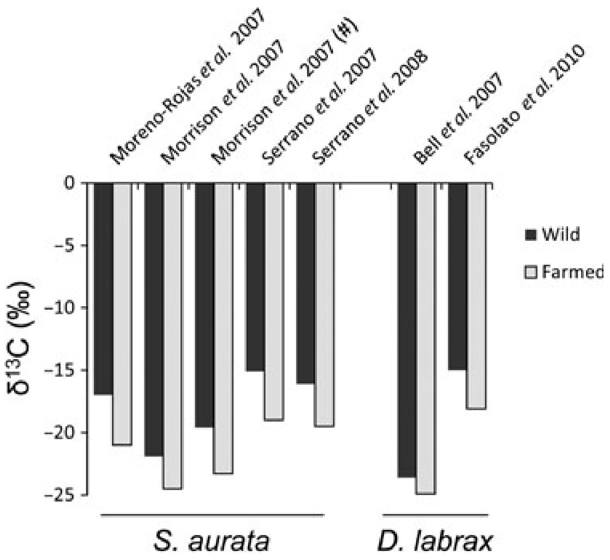


Figure 3 Literature review of δ^{13}C isotopic composition on sea bream and sea bass fish muscle with significant differences. Percentages of total FAs of wild fish (black bars) and farmed fish (grey bars). All results are δ^{13} of the bulk oil fraction of flesh total lipids; except (#) that is from the glycerol/choline concentrated fraction from flesh lipid extract.

Furthermore, analysis of δ18O from the total oil extracted from flesh lipid on sea bream exhibited significant differences between farmed and wild fish (Morrison *et al.* 2007). In contrast, analysis of δ18O in sea bass muscle did not show significant differences (Bell *et al.* 2007). However, analysis of δ18O may reflect latitudinal differences in the mean ocean δ18O, which is in isotopic equilibrium with fish metabolic water and therefore may discriminate between fish caught in different geographical locations (Bell *et al.* 2007; Morrison *et al.* 2007). The utility of using δ18O as a discriminatory factor between farmed and wild fish across species must be considered carefully. While the geographical location of farmed fish is controlled, wild fish may migrate over large geographical regions (Bell *et al.* 2007; Morrison *et al.* 2007). The utility of compound-specific isotope analysis has been suggested as a powerful tool to discriminate wild and farmed sea bream and sea bass. Particularly, individual fatty acid δ13C and bulk δ13C are good indicators of farmed or wild fish origin. Standardization of different methodologies is required to provide a robust method able to be applied consistently over a range of species from both the aquaculture and fisheries sectors.

Trace elements

Marine fish incorporate different trace elements from the environment into their skeletal tissues and organs, either present in seawater or the diet, forming a chemical signature that will reflect the length of time that a fish has inhabited a particular water body (Lal 1989). Hence, trace element profiles are likely to be unique to a given population that inhabits one given location. Wild populations of sea bass and sea bream in the Mediterranean move among various coastal habitats, thus it is difficult to detect substantial differences in trace elemental signatures among different populations of wild fish (Gillanders *et al.* 2001). However, aquaculture creates a special situation in which fish are static in one location with unique environmental conditions. Under such circumstances, a distinct trace element composition is likely to appear (Patterson & Kingsford 2005). Where cage farming exists, in addition to the natural presence of metals in the aquatic environment from geochemical and anthropogenic processes, extra sources of trace metals may result from metal-based antifoulants used periodically to protect the nets from fouling, such as Cu. Fish diets are also enriched with various essential metals, including Cu, Fe, Zn, Mn, Co, Cr and Mg among others (CIESM 2007).

Therefore, differences between farmed and wild sea bream and sea bass have been found through trace element analysis (see Table 5). However, contrasting results have been reported within the reviewed literature. In sea bream, the muscles of wild fish were found to be more polluted than farmed fish, presenting significantly higher values of Hg and As (Minganti *et al.* 2010), with either discrepancies or no significant differences on other studied elements being reported (Carpene *et al.* 1998; Minganti *et al.* 2010). Regarding trace elements in otoliths, farmed sea bream presented higher values of Ba, Mn and Fe, and lower of Sr, than their wild conspecifics (Sanchez-Jerez, unpubl. data, 2012). The calcified tissues do not incorporate trace elements in the same way, since otoliths 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). The trace metal microchemistry of salmon scales has been used successfully to discriminate between farmed and wild fish (Adey *et al.* 2009) and differences were also seen between farmed fish that shared the same water body and diet – presumably caused by subtle differences in local water chemistry. Recent research within the Prevent Escape project shows that a similar analysis effectively discriminates wild and farmed sea bass and sea bream in the Mediterranean (Black, unpubl. data, in Sanchez-Jerez 2012). In sea bass, reviewed trace elements analysis in muscle and liver showed considerable divergence among the results. However, it seems that wild sea bass contain a higher proportion of heavy metals, such as Hg and Pb, probably as a consequence of pollution of the seawater by industrial discharges (Monti *et al.* 2005). It is remarkable that toxic elements, which have been considered to be detrimental to humans if

Table 5 Resulting trace elements on different tissues for sea bream and sea bass from the literature reviewed

Author	*Tissue*	Na	K	Mg	Ca	Sr	Ba	Ti	V	Cr	Mo	Mn	Fe	Co
Sparus aurata														
Black (u.d.)	S.	–	–	NS	–	W>F	W>F	NS	W>F	F>W	F>W	NS	F>W	NS
Carpene *et al.* (1998)	M.	–	–	–	–	–	–	–	–	–	–	–	NS	–
Minganti *et al.* (2010)	M.	–	–	–	–	–	–	–	NS	NS	NS	NS	NS	–
Sanchez-Jerez (u.d.)	O.	–	–	NS	–	W>F	F>W	–	–	–	–	F>W	F>W	–
Dicentrarchus labrax														
Alasalvar *et al.* (2002a)	M.	–	–	–	–	–	–	W>F	F>W	NS	NS	NS	W>F	NS
Black (u.d.)	S.	–	–	NS	–	F>W	W>F	NS	F>W	NS	NS	F>W	NS	NS
Fernandes *et al.* (2007)	M., L.	–	–	–	–	–	–	–	–	–	–	–	–	–
Ferreira *et al.* (2010)	M.	–	–	–	–	–	–	–	–	–	–	–	–	–
Ferreira *et al.* (2010)	L.	–	–	–	–	–	–	–	–	–	–	–	–	–
Fuentes *et al.* (2010)	M.	NS	NS	NS	NS	–	–	–	–	–	–	NS	NS	–
Mnari *et al.* (2010a)	dM.	NS	F>W	NS	F>W	–	–	–	–	–	–	F>W	W>F	–
Mnari *et al.* (2010a)	vM.	W>F	W>F	W>F	W>F	–	–	–	–	–	–	F>W	W>F	–
Mnari *et al.* (2010a)	L.	W>F	W>F	W>F	F>W	–	–	–	–	–	–	NS	W>F	–
Monti *et al.* (2005)	M.	–	–	–	–	–	–	–	NS	NS	–	F>W	NS	W>F
Orban *et al.* (2002)	M.	NS	NS	NS	NS	–	–	–	–	NS	–	–	F>W	–
Sanchez-Jerez (u.d.)	O.	–	–	NS	–	NS	F>W	–	–	–	–	F>W	NS	–
Santaella *et al.* (2007)	M.	W>F	W>F	NS	NS	–	–	–	–	–	–	–	W>F	–
		Ni	Cu	Ag	Zn	Cd	Hg	Al	Pb	B	As	Sb	P	Se
Sparus aurata														
Black (u.d.)	S.	F>W	F>W	NS	NS	NS	–	F>W	W>F	–	W>F	–	–	NS
Carpene *et al.* (1998)	M.	–	NS	–	NS	–	–	–	–	–	–	–	–	–
Minganti *et al.* (2010)	M.	–	NS	–	NS	–	W>F	–	–	–	W>F	–	–	–
Sanchez-Jerez (u.d.)	O.	–	–	–	–	–	–	–	–	–	–	–	–	–
Dicentrarchus labrax														
Alasalvar *et al.* (2002a)	M.	NS	NS	NS	NS	NS	–	W>F	NS	–	–	–	–	–
Black (u.d.)	S.	NS	NS	NS	NS	NS	–	W>F	W>F	–	W>F	–	–	W>F
Fernandes *et al.* (2007)	M., L.	–	F>W	–	NS	F>W	–	–	–	–	–	–	–	–
Ferreira *et al.* (2010)	M.	–	F>W	–	–	NS	–	–	NS	–	NS	–	–	–
Ferreira *et al.* (2010)	L.	–	NS	–	–	W>F	–	–	W>F	–	W>F	–	–	–
Fuentes *et al.* (2010)	M.	–	NS	–	NS	–	–	–	–	–	–	–	NS	–
Mnari *et al.* (2010a)	dM.	–	W>F	–	F>W	–	–	–	–	–	–	–	–	–
Mnari *et al.* (2010a)	vM.	–	NS	–	F>W	–	–	–	–	–	–	–	–	–
Mnari *et al.* (2010a)	L.	–	F>W	–	W>F	–	–	–	–	–	–	–	–	–
Monti *et al.* (2005)	M.	NS	F>W	–	W>F	W>F	W>F	W>F	F>W	F>W	NS	NS	NS	NS
Orban *et al.* (2002)	M.	–	–	–	F>W	–	W>F	–	–	–	–	–	NS	W>F
Sanchez–Jerez (u.d.)	O.	–	–	–	–	–	–	–	–	–	–	–	–	–
Santaella *et al.* (2007)	M.	–	–	–	W>F	–	–	–	–	–	–	–	F>W	–

M, muscle; O, otoliths; S, scales; L, liver; d, dorsal; v, ventral; F, farmed fish; W, wild fish; NS, no significant differences.

ingested in concentrations above certain levels, were present at levels below their hazard level in all reviewed studies. Interestingly Cu, which is below the detection limit in the studied wild fish, was present in a measurable amount in farmed fish, probably from anti-fouling components (Monti *et al.* 2005; Ferreira *et al.* 2010). Through scales and otoliths, farmed sea bass showed higher values of Mn and Ba, and lower Sr, than wild sea bass. Despite the fact that studies gave contrasting results, most were able to distinguish wild and farmed fish with great accuracy, but only through a multivariate approach that accounted for a wide range of elements. No simple diagnostic elemental concentration or ratio exists. This indicates that the trace elemental profile might be more appropriate than the presence or absence of a specific quantity of an element.

Accumulative pollutants

Marine organisms can be contaminated by potential exposure to various pollutants such as organochlorine compounds (OCs) and heavy metals, resulting in most of the cases of the direct consumption of feed or from the surrounding environment (Grigorakis & Rigos 2011). These OCs are represented mainly by polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polybrominateddiphenyl ethers (PBDEs), hexachlorobenzenes (HCBs), dichlorodiphenyltrichloroethane (DDT) and its degradation product (DDE) (Antunes & Gil 2004; Ferreira *et al.* 2010; Grigorakis & Rigos 2011). The organochlorines are lipophilic or hydrophobic compounds that accumulate preferentially in adipose tissues, therefore, the higher fat content of farmed fish indicates that they may have a larger reservoir for absorption of such persistent contaminants compared with their wild counterparts (Grigorakis & Rigos 2011).

Several studies have documented the occurrence of various organochlorines in aquaculture products and feed by providing useful comparisons between wild and farmed fish in both sea bream and sea bass species in the Mediterranean. Concerns were initially raised with the report of higher pollutant levels (e.g. PCBs, toxaphene or dieldrin) for salmon aquaculture (Hites *et al.* 2004). However, farmed sea bream have lower levels of pollutants in their tissues than wild sea bream, with both groups always containing levels below the guidelines recommended for human consumption (Serrano *et al.* 2008; Blanes *et al.* 2009). Total PCBs and DDTs in the muscle, liver and gills are higher in wild fish than in cultured sea bream, with the highest concentration of pollutants found in tissues of higher lipid content, despite the higher biomagnification factors found in farmed specimens (Serrano *et al.* 2008; Blanes *et al.* 2009). The concentration of OCs is uniform throughout the year in farmed sea bream, while wild fish showed higher seasonal variability, reflecting environmental factors and seasonality in growth rates. The low levels of contaminants found in the feed supplied to farmed sea bream explain the organochlorine concentrations in their tissues which remain below those of wild fish, in spite of the intensive culture conditions and the higher trophic level of cultured specimens (Serrano *et al.* 2008; Blanes *et al.* 2009).

In contrast, farmed sea bass are more contaminated than wild sea bass, but their levels were also below those recommended for human consumption. Wild specimens have lower levels of total PCBs and DDTs, both in the liver and muscle, compared with cultured bass (Antunes & Gil 2004; Carubelli *et al.* 2007; Fernandes *et al.* 2007; Lo Turco *et al.* 2007). Similarly, Ferreira *et al.* (2010) found a higher accumulation of OCs in cultured sea bass in the Eastern-Atlantic, but lower concentrations of metals compared with wild bass. Higher exposure to PAHs was also observed in wild sea bass (Fernandes *et al.* 2007). As for sea bream, these differences have been attributed to the level of contaminants present in the diet and different feeding characteristics due to the intensive culture in the farms (Antunes & Gil 2004; Serrano *et al.* 2008). Therefore, this technique is likely to be more related to the feeding history of the fish and/or to the level of contamination of the site where the fish was farmed or caught.

Genetic differences

The intensification of fish culture over recent decades has increased the need for the mass production of good quality eggs or/and fingerlings to supply aquaculture. Often, the genetic structure of cultivated populations differs from wild populations and this can be used to identify the origin of fish. Genetic differences between cultured and wild fish can be apportioned across three hierarchical levels, where the upper level includes the differentiation from the previous levels. The first level of differentiation is due to founder effects and is common for most cultured organisms. Broodstock are captured from the wild, but represent a small sample of the whole population. Thus, they only carry a small proportion of the genetic diversity of the whole population. This founder effect is often accompanied by the loss of rare alleles and changes in allele frequencies. Furthermore, the effective population size is lower in cultured populations than in the wild (Youngson *et al.* 2001). A slight decrease of variability of genetic structure in cultivated populations compared with wild stocks has occurred for sea bream (Palma *et al.* 2001; Alarcón *et al.* 2004).

The second level of differentiation occurs when the farmed stock comes from a different location and is genetically different from the local wild population. Sea bream and sea bass hatcheries export eggs or fingerlings to fish farms in several countries around the Mediterranean (Youngson *et al.* 2001; Haffray *et al.* 2007). Therefore, the genetic differentiation between the wild and farmed populations depends on the differences between local populations and the population from which the farmed fish originated. Wild population genetic structure is well studied in European sea bass. Three genetically distinct zones exist: the Atlantic Ocean (the Alboran Sea included), the western Mediterranean and the eastern Mediterranean (Patarnello *et al.* 1993; Allegrucci *et al.* 1997; Garcia de Leon *et al.* 1997; Castilho & McAndrew 1998; Sola *et al.* 1998; Bahri-Sfar *et al.* 2000, 2005; Castilho & Ciftci 2005; Ergüden & Turan 2005; Katsares *et al.* 2005; Lemaire *et al.* 2005). Microsatellite markers have been used to detect cases where (mostly eastern Mediterranean) population samples did not cluster according to their geographical origin, but with western Mediterranean samples (Patarnello *et al.* 1993; Allegrucci *et al.* 1997). Therefore, it is evident that some wild stocks may have already been affected by escapees (Bahri-Sfar *et al.* 2005).

Sea bream display a different pattern of genetic differentiation to sea bass. Some studies on wild sea bream show a slight genetic differentiation between Mediterranean populations, but this was not apparently associated with geographical or oceanographical factors (Palma *et al.* 2001; Youngson *et al.* 2001; Alarcón *et al.* 2004; Ben-Slimen *et al.* 2004; De Innocentiis *et al.* 2004; Rossi *et al.* 2006). However, De Innocentiis *et al.* (2004) identified a genetic differentiation between Atlantic and Mediterranean wild populations, and further fine-scale differentiation within the Mediterranean Sea. Despite such discrepancies, the migration of individuals and consequent crossing between neighbouring populations seems to be the main reason for the slight genetic differentiation found (Palma *et al.* 2001), but justifies the difference between local wild sea bream populations and adjacent farmed populations.

The third level of differentiation is the effect of artificial selection through selective breeding of cultured fish. When specific characteristics are selected artificially, the genetic structure of the genome may change due to the hitchhiking effect. However, dramatic changes in phenotype do not occur rapidly. Sea bass and sea bream are selectively bred (Garcia de Leon *et al.* 1998; Brown 2003) for commercially desirable traits such as high growth rates, disease resistance, absence of skeletal malformations, altered aggression and adaptation to high stocking densities (Boglione *et al.* 2001; Thorland *et al.* 2006; Dupont-Nivet *et al.* 2008; Antonello *et al.* 2009; Navarro *et al.* 2009; Grigorakis & Rigos 2011). In this process, little attention is given to possible impacts on the genetic integrity of wild stocks when farmed fish escape (Miggiano *et al.* 2005). Therefore, new alleles or allelelic combinations from farmed fish (adults or eggs escapes) could invade the local population and change its genetic structure through introgression. Although the impact of mixing of farmed and natural populations has not been assessed, theory suggests that captive-bred organisms could reduce the fitness of wild populations (Lynch & O'Hely 2001; Ford 2002; Araki *et al.* 2009). The genetic structure of a local population is the result of long-term interactions between the population and the environment. Different environments might favour different alleles or allelic combinations which maximize fitness populations in specific environments. Genetic drift might also have fixed different alleles in different populations. Furthermore, populations that have undergone artificial selection could be less fit in natural environments. Thus, introducing farmed genotypes into local wild populations could reduce the fitness of wild individuals. In addition, the large differences between natural spawning and hatchery conditions involve the risk of inbreeding, or causing artificial gene flow, which could induce a biodiversity decline or outbreeding depression (Sola *et al.* 1998).

Differences in the genomic structure of farmed and wild populations can be used to develop genetic tags for the identification of escapees. Moreover, genetic tagging of sea bream and sea bass broodstocks in commercial hatcheries might be a suitable tool to monitor the genetic impact of fish farm escapes and/or restocking releases (Castro *et al.* 2007; Sola *et al.* 2007). The advantage of genetic methods is that they can be performed at all life stages; from egg to adult. The accuracy of the 'DNA stand-by method' (Glover *et al.* 2008, 2009a,b) for the identification of escapees depends on the degree of divergence of farmed from wild populations. This method has been used successfully to identify escapees in a number of species such as cod, salmon and trout (Glover *et al.* 2008, 2010), but has not been applied for sea bream or sea bass. Recent studies show that sea bream and sea bass farmed populations are significantly different from wild populations in the Mediterranean (Karaiskou *et al.* 2009; Loukovitis *et al.* 2012). Thus, the 'DNA stand-by method' could be applied to identify fish farm escapees for a wide range of aquaculture species in all regions of the world (Glover 2010).

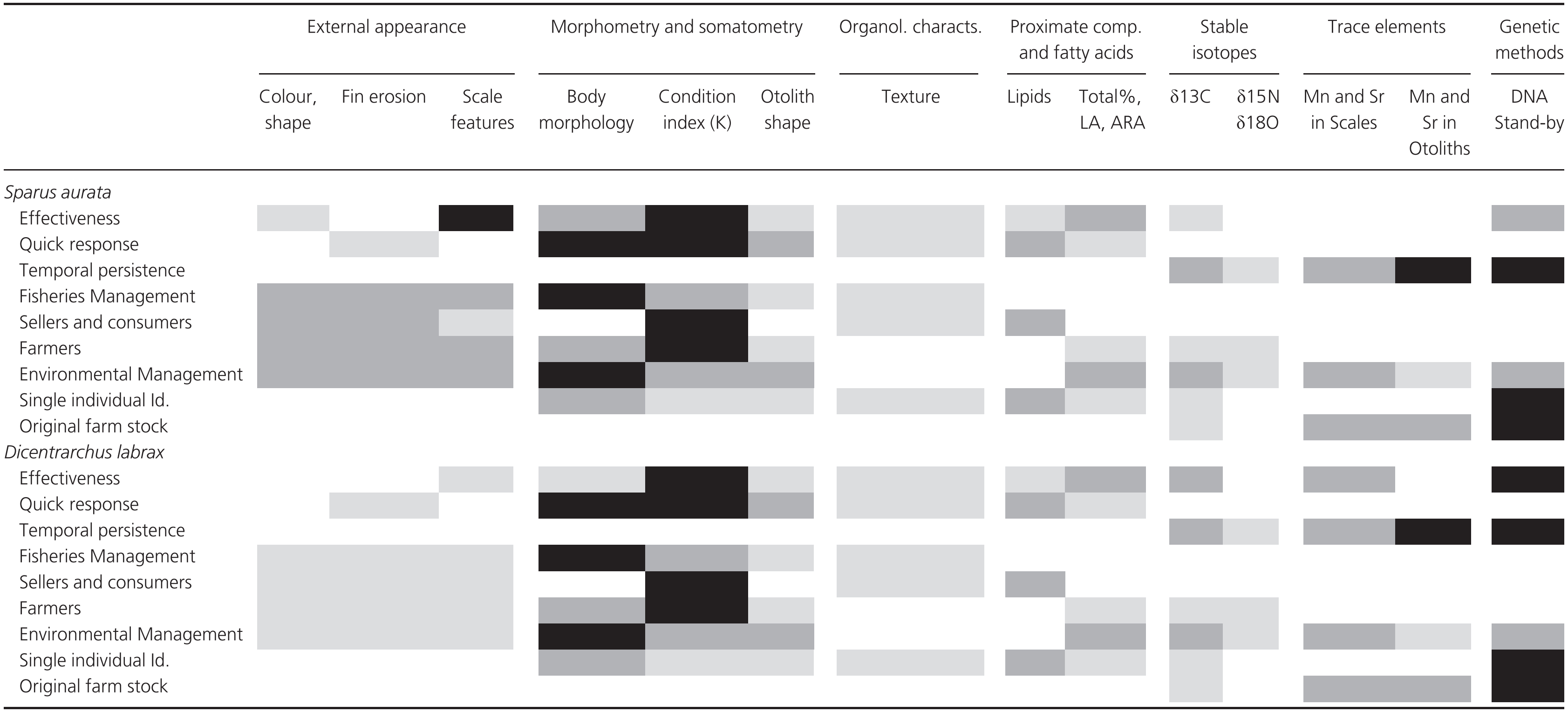
Implication and guidelines

Few studies have sought to develop techniques with the purpose of identifying escaped farmed sea bream and sea bass within wild populations to assist in determining their genetic and ecological effects. To evaluate the potential risk to wild stocks due to farmed escapees and to determine the contribution of escapees to fisheries landings, robust fish-origin indicators are required. Sanchez-Jerez (2012) used a Delphi-method, which is based on questionnaires sent to experts, to score different indicators according to their effectiveness, ability to be used quickly, accuracy, applicability and usefulness for different sectors. This Delphi study and the knowledge summarized in the present review enable us to recommend indicators to detect escaped sea bream and sea bass in wild populations (see Table 6).

Morphology and external appearance are highly useful to distinguish farmed from wild fish for both sea bream and sea bass. Further, the use of external characteristics (e.g. colour patterns, fin erosion, scale features), combined with morphometry (e.g. body and otolith shape, condition index) or/and organoleptic descriptors (e.g. textural parameters), are the easiest, quickest and cheapest ways to discriminate the wild or farmed origin of fish but their use is limited only to early escaped fish (Tables 2,6). These techniques can be used directly in the field with high accuracy (e.g. scales), without laborious or expensive equipment, and they do not require expert knowledge or specialization. Organoleptic characteristics are likely to be less useful in this regard as an expert panel is required accurately to assess such descriptors. Therefore, we recommend external characteristics and morphology as a means to detect escapees immediately post-escape for farmers, fishermen, sellers, consumers, scientists and managers.

How long these parameters persist post-escape, which will influence their accuracy, remains unknown. Hence, we recommend the application of a holistic and multidisciplinary approach using other physico-chemical parameters for research and management applications that require tracing escapes for time scales greater than several months. Chemometric tools can discriminate wild and farmed origin for

Table 6 Colour table summarizing the recommendations for applicability of indicators of sea bream and sea bass escapes concerning to different aspects. Range of colour corresponds from more accuracy or recommended (black) to less accuracy or recommended (white) (modified from Sanchez-Jerez 2012)



	External appearance			Morphometry and somatometry			Organol. characts.	Proximate comp. and fatty acids		Stable isotopes		Trace elements		Genetic methods
	Colour, shape	Fin erosion	Scale features	Body morphology	Condition index (K)	Otolith shape	Texture	Lipids	Total%, LA, ARA	δ13C	δ15N δ18O	Mn and Sr in Scales	Mn and Sr in Otoliths	DNA Stand-by
Sparus aurata														
Effectiveness	░		■	▓	■	░	░	░	▓	░				▓
Quick response		░		■	■	▓	░	▓	░					
Temporal persistence										▓	░	▓	■	■
Fisheries Management	▓	▓	▓	■	▓	░	░							
Sellers and consumers	▓	▓	░		■		░	▓						
Farmers	▓	▓	▓	▓	■	░			░	░	░			
Environmental Management	▓	▓	▓	■	▓	▓			▓	▓	░	▓	░	▓
Single individual Id.				▓	░	░	░	▓	░	░				■
Original farm stock										░		▓	▓	■
Dicentrarchus labrax														
Effectiveness			░	░	■	░	░	░	▓	▓		▓		■
Quick response		░		■	■	▓	░	▓	░					
Temporal persistence										▓	░	▓	■	■
Fisheries Management	░	░	░	■	▓	░	░							
Sellers and consumers	░	░	░		■		░	▓						
Farmers	░	░	░	▓	■	░			░	░	░			
Environmental Management	░	░	░	■	▓	▓			▓	▓	░	▓	░	▓
Single individual Id.				▓	░	░	░	▓	░	░				■
Original farm stock										░		▓	▓	■

sea bream and sea bass (Table 6). Specifically, the analysis of total lipids and FA profiles (mainly the proportional presence of LA and ARA), the proportion of δ13C in fish flesh, and the presence of specific trace elements (such as Cu and Hg in fish flesh and Mn and Sr in otoliths and scales), are likely to reflect the different environmental and feeding characteristics of fish. Moreover, several authors have demonstrated the usefulness of a multivariate approach, which draws upon information derived from diverse techniques, as the best way to discriminate fish origin and escapees with high accuracy. However, such techniques are usually expensive and require specific knowledge, making them unavailable to many sectors.

Fish physiology and biochemical process are strongly influenced by geography and seasonal variability, and limits to both the detection and discrimination of chemical characteristics should be established to create analytical and management protocols which can be applied widely. In the future, new chemical or molecular techniques may be developed that allow the discrimination of escapees with higher accuracy (e.g. the use of unique chemical otolith markers in juvenile fish from hatcheries). Finally, the use of molecular genetic markers is likely to be a suitable tool for genetic discrimination of wild and farmed fish, enabling monitoring of the genetic impact of fish farm escapes and/or restocking releases (Table 6). Broodstock and their offspring should be genotyped in hatcheries before going to open-sea cages, and such information should be available to the scientific community and managers, in order to improve the accuracy and suitability of genetic tools. Unfortunately, genetic techniques, in spite of being non-destructive and being highly informative, are among the most expensive and time-consuming techniques currently available.

A wide range of fish origin descriptors are available to detect escapees within wild stocks. Some can be applied easily to evaluate the contribution of escapees to other sectors, such as fisheries, markets and consumers, making them useful for rapid assessments which do not require 100% accuracy. As numerous tools can be used to achieve specific outcomes, we recommend that regulatory bodies must establish protocols through legislation that specify the most appropriate techniques for each particular circumstance.

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