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IDENTIFICATION OF BIOMARKERS IN FISHES ASSOCIATED TO CAGE AQUACULTURE

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Trabajo realizado en el Instituto canario de ciencia marinas (ICCM) de las palmas de Gran Canaria, España bajo la dirección del Dr. Ricardo Harun Tabraue y Dr. Daniel Montero Vitores.

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Abstract

During the last decades, the increasing fish-farming activity in coastal areas have lead to a larger amount of nutrient wastes discharged into the water column and nearby sediments, which may dissipate or, alternatively, enter coastal trophic chains. The study was done in the Canary Islands (Central Eastern Atlantic), which are characterized with exposed and oligotrophic waters, at several distances from 2 different fish farms rearing Sea bass (*Dicentrarchus labrax*). The selected biomakers studied were fatty acid profiles, body and scales morphometries and otolith weight and otolith shape analysis. Four fish species representing different trophic guild levels were analyzed: a carnivorous/predator (Yellowmouth barracuda: *Sphyraena viridensis*), a herbivor (Salema: *Salpa salpa*), a sediment-feeder (Surmullet: *Mullus surmuletus*) and a plankton-feeder (Bogue: *Boops boops*). In this contribution, we shall discuss the main results obtained by analyzing the efficiency of those biomakers as a tool to differentiate fishes associated to aquaculture farms comparing them with wild fishes and fishes associated to seawage.

Key words: Biomarker, Fatty acid profile, body morphometry, scale morphometry otolith morphometry,otolith weight, aquaculture.

Introduction

1. Importance of aquaculture production activity in the world

The aquaculture activity represents nowadays one of the fastest growing animal food producing sectors of the last decades which has grown at an average rate of 8.1% per year since 1960. Nowaday, this sector account for nearly 50% of the world's food fish (FAO, 2009, 2010) (Figure 1).

This increase was part of a challenge, not only to adequately feed the population of the world but also to improve their quality of life providing them with better animal protein at competitive prices, and this could only be achieved by developing the aquaculture sector, because fisheries are not able to cope with the world demands.

Efforts have been spent to fulfill this purpose and resulted in a better aquaculture production. Global production of farmed fish and shellfish has more than doubled in the past 15 years in addition aquaculture increased from 12 million metric tons in 1985 to 45 million metric tons by 2004 (Diana, 2009). The tremendous development of the aquaculture sector was surely made through the introduction of new technologies, and a better understanding of the biology of the farmed species (Read & Fernandes., 2003).

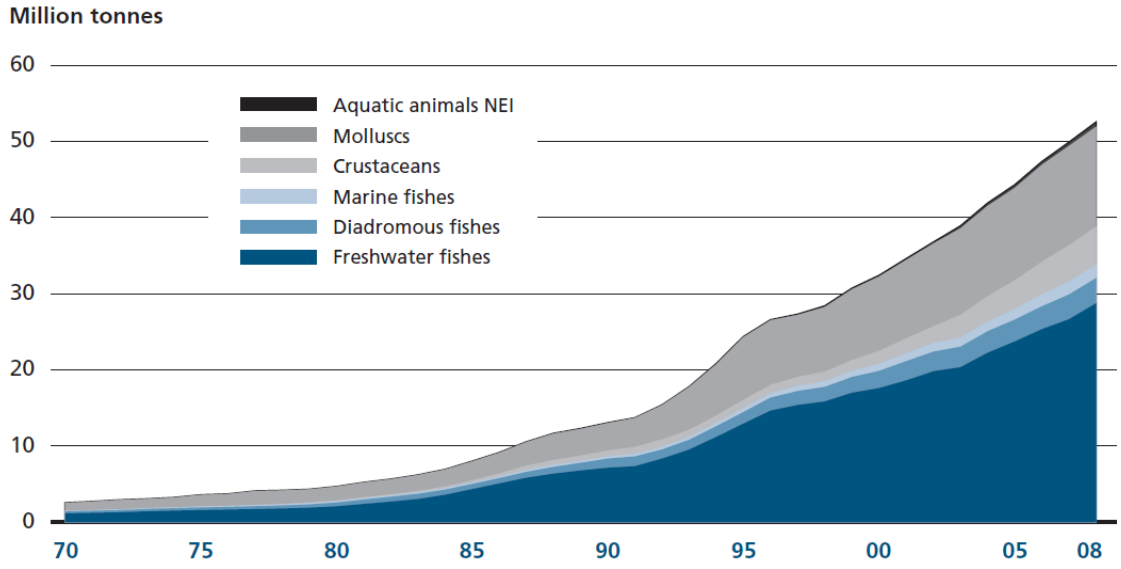


Figure 1. Trends in world aquaculture production: major species groups (FAO, 2010)

This fast development of the aquaculture sector was done with an increasing demand for exploitation of natural resources, including clean water and adequate sites (Read & Fernandes, 2003) as well as fish meal and fish oil consumption as primary ingredients for aquafeeds due to its high nutritional value in diets for animals (Tacon & Metian, 2008). Aquaculture constitutes the fastest growing food production sector and the main contributor of marine food to satisfy the demand of the unceasingly increasing human population (Tacon & Metian, 2008).

As consequences of its rapid growth rate, aquaculture has faced new environmental problems. Since the supply of these resources is limited, the increasing demand from the aquaculture sector have enhanced the competition with other users for the same resources and generated significant public concern over environmental issues (Karakassis et al., 2000; 2005).

2. Interaction of Aquaculture and Environment

The marine aquaculture performed in the sea, is indeed, closely related to its environment as it uses the natural marine water as a vector of the introduction of energy through the aliment, but also a vector of its dissipation, as water allows the release of wastes into the environment. This interaction between aquaculture and environment could be at a certain point beneficial but also damaging to the ecosystem.

2.1 Environmental effects from the presence of aquaculture cages

2.1.1 Beneficial environmental effect

Fish farms have a strong aggregative effect on wild fishes (Dempster et al., 2002) and the aggregation of wild fishes around or near fish farms could be beneficial to wild fish stocks. In addition, the wild fishes contribute to the consumption of the persistent supply of unused artificial food coming from farms which may enhance their growth.

The presence of aquaculture may on one hand increase fisheries resources in the wider area of an aquaculture zone (Machias et al., 2003, 2004). In addition, to increase production of local fisheries through the aggregation of wild adults around the aquaculture farms (McClanahan & Mangi, 2000; Fernandez-Jover et al., 2008) which increase the spawning-stock biomass and may amplify the larval recruitment (Chiappone& Sullivan, 2000).

On the other hand, the pools of wild fishes around sea cages act as potential organic waste-consumers in the water column as well as in the sediment (Fernandez-Jover et al., 2008).

This bio-filtering process contribute to the recycling of organic matter of the sediment and regulating the benthic community structure (Vita et al., 2004). As a result feeding of wild fish around sea cages may diminish the amount of food that reaches the sea floor and reduce effects upon the benthos (Katz et al., 2002).

2.1.2 Drawbacks

2.1.2.1 Habitat modifications

The aquaculture activity has participated to modificate habitats of many coastal regions in the world (Islam, 2005); hundreds of thousands of hectares of mangroves and coastal wetlands around the world have been transformed into milkfish and shrimp ponds with losses of essential ecological services that mangroves provide, such as protection of the coast from battering storms and typhoons, flood control, trapping of sediments, and filtering and cleansing of nutrients from the water (Naylor et al., 2001).

2.1.2.2 Escaping and genetic alteration

Escapement from cages is almost inevitable in all aquaculture system (Arthur et al., 2010) The potential of many aquaculture species to become invasive after escaping is high (Diana, 2009) because they usually have a widely distributed original range, a broad environmental tolerance, high genetic variability, short generation time, rapid growth, and early sexual maturation (Vila-Gispert et al., 2005). Those attributes could lead to undesired effects on wild population by introducing new species or modified genotypes (Thorstad et al., 2008). For instance, Crozier (1993) demonstrated an interbreeding between the farmed Salmon and the wild population in the Glenarm Bay in Northern Irland (Hindar et al., 2006). As they differ genetically from wild populations due to the domestication selection traits and genetic drift (Ferguson et al., 2007). This interbreeding threaten the genetic integrity of wild salmon populations (Glover et al., 2009).Besides, escaping accident occurs frequently, it was reported that in Norway, 3.93 million Atlantic salmon *Salmo salar*, 0.98 million rainbow trout *Oncorhynchus mykiss* and 1.05 million Atlantic cod *Gadus morhua* escaped from 2001 to 2009 (Jensen et al., 2010).

2.2 Aquaculture Cages Attracts Wild Fish

Aquaculture interacts with wildlife through its consumption of resources, the aquaculture process itself and through the release of wastes into the environment, especially feral fish (Beveridge, 2000; Islam, 2005). The presence of aquaculture cages in the sea can indeed

interfere with the natural ecosystem as it has been studied that the large concentrations of fish present into the cages together with the food available for their alimentation can be appealing for certain predators and scavengers (Figure.2) (Beveridge, 2001; Machias et al.,2004, 2005& 2006; Pitta et al., 2009).

Figure 2. Wild fish *Pollachius virens* aggregate around aquaculture cages in Norway (From Dempster et al., 2009)

Diverse range of predatory species has been observed, not only reptiles and birds (Littauer et al., 1997) but also mammals (Wursig & Gailey, 2002) in areas nearby to aquaculture farms which lead to an increasing predation and competition for breeding sites. Meanwhile the aggregation of wild fishes around sea cages has been the subject of a several studies (Dempster et al., 2002 ,2004, 2009&2011; Thetmeyer et al., 2003; Tuya et al., 2006; Valle et al., 2007; Sudirman et al., 2009), focusing on different aspects of wild fish populations, such as fish abundance or biomass around the cages. It was demonstrated that fish aggregation around cages depend not only on environmental conditions, such as coastal morphology, distance to the coast, currents, and depth, farm characteristics (Fernandez-Jover et al., 2008) but also on the quantity of feed lost from the cages (Dempster et al., 2009).

This attraction is due to the aquaculture infrastructure formed by cages and raft structures acting as fish attractant devices (FADs), (Rountree, 1989; Carss, 1990; Bjordal & Skar, 1992; Dempster et al., 2002, 2011) which offer shelter to some species and habitat for predators described around sea cage fish farms in several temperate and tropical locations (Sanchez-Jerez et al., 2008), Greece (Smith et al., 2003;Thetmeyer et al., 2003), the Canary Islands (Boyra et al. 2004, Tuya et al., 2005; Tuya et al., 2006), Australia (Dempster et al., 2004) and Indonesia (Sudirman et al., 2009).

2.2.1 Case of Norway

Norway is an important Salmon producer, almost 689.000 t of salmonids have been produced from 1198 coastal farms in 2007 (Jensen et al., 2010). Howerver, this activity precipitates ecological changes in coastal ecosystems through the increasing of nutrient inputs that modifies the benthic communities. Besides the escapes of farmed fish that is affecting the wild salmonid populations (Heggberget et al., 1993; Weir & Grant, 2005; Hindara et al., 2006). In addition to that, wild fish population has been observed aggregating around aquaculture cages, this was the subject of a study of Dempster and co-workers (2009) where it was estimated that over 12. 000 t of wild fish aggregated into a total of 750 ha of coastal waters on any given day in summer where Gadids dominated the farm-associated assemblages in both number and biomass (*Pollachius virens*, *Gaus morhua* and *Melanogrammus aeglefinus*), while *Scomber scombrus* were common in surface waters around farms in southern Norway (Valle al., 2007).

2.2.2 Case of Greece

In the Aegean waters of Greece, Machias and co-workers (2005) demonstrated that the presence of fish farms affected the benthic community structure (Neofitou et al., 2010) and also the species composition, abundance and diversity of demersal fish assemblages in the bay of Petalioi in the South Evoikos Gulf (South Aegean Sea). After the establishment of aquaculture cages, the overall abundance of the fish assemblage increased by a factor of 4 and the average trophic level of the fish community increased from 3.59 to 3.79 (Machias et al., 2004).

2.2.3 Case of Canary Islands

In the Canary Islands, it was reported that important wild fish population were assembling around aquaculture cages. In this context, Tuya and co-workers (2005), compared three coastal habitats: 1) Unvegetated sandy substrates with no overlying fish farm, 2) Vegetated seagrass beds; and 3) Sandy bottoms beneath the sea-cage fish farms, in three islands of the Canarian Archipelago separated between 60 km and 200 km. This study has shown the attraction and increase in the abundance of a certain group of bentho-demersal wild fish populations to soft bottoms beneath sea-cage fish farms. The overall fish abundance is dominated by the ostheichthyes *H. longissimus* and *P. acarne*, as well as to a group of large-sized benthic chondrichthyes (*T. grabata*, *M. aquila* and *G. altavela*).

In another study performed by Dempster and co-workers (2005) about fishes associated to aquaculture cages in Canary Island farms, 32 fish species were observed. The most common families were Sparidae (8 species) and Carangidae (6 species), with Chondrichthyid rays also common (7 species). Only 3 species (*Boops boops*, *Pagellus acarne* and *Synodus saurus*) occurred at both the Mediterranean and Canary Island farms. Assemblages were numerically dominated by planktivorous or food-pellet feeding species (*Sardinella aurita*, *B. boops*, *T. mediterraneus*, Mugilidae, *T. ovatus*), a number of large piscivores (*Pomatomus saltatrix*, *Sphyraena sphyraena*, *Sphyraena viridensis*, *Coryphaena hippurus*, *Auxis rochei*, *Sarda sarda*). In addition to the ichthyofauna, mammals also were observed and represented by the bottlenose dolphin *Tursiops truncatus*. This was later confirmed by Tuya and co-workers (2006) who demonstrated that beneath a floating sea-cage fish farm in Gran Canaria Island, the aggregative effect on wild fishes compared to nearby controls decreased from c. 50 times when the farm was in full operation to less than two times when only the farm structures remained.

2.3 Release of uneaten feed and feces

Humans rely on the assimilative capacity of waters as an essential ecosystem service (Silvert, 1992). In fact, nutrient loading through discharges of fish wastes and uneaten feed coming from aquaculture cages are discharged in the water because they are considered as assimilated into primary or secondary production (Figure 3) (Diana., 2009) this contribute to the nutrient pollution near coastal fish ponds and cages and depend strongly on the specific hydrography of the water (Neylor et al., 2001), the impact is stronger when the wastewaters are situated in confined water communities (Gyllenhammar& Hakanson,

2005). In such areas, buildup of food particles and fecal pellets under and around fish pens and cages interferes with nutrient cycling in seabed (Beveridge, 2001).

2.3.1 Aquaculture wastes and the water quality

The wastewater discharged from intensive aquaculture into coastal water may lead to deterioration in water quality. Waste produced by fish farms contains carbon, phosphorus and nitrogen in dissolved and suspended solids (Figure 3) (Naylor et al., 2001; Islam, 2005) as well as trace metals, such as zinc and copper (Beveridge, 1996; Aich et al., 2012). Depending on the species and culture techniques, up to 85% of phosphorus and 52±95% of nitrogen input into a marine fish culture system as feed may be lost into the environment through feed wastage, fish excretion, faeces production and respiration (Tovar et al, 2000).These nutrient loadings can boost primary production and lead to water eutrophication (Tovar et al, 2000). The discharge of waste from a fish cage is primarily in the form of dissolved nutrients (60% of the food supplied of the cages) (Karakassis et al., 2005), at least half of which are nutrients that are immediately available to phytoplankton. This trophic perturbation is responsible for the changes in the trophic status by increasing (Neophitou et al., 2010) by changes in demersal fish community and macrobenthic population (Machias et al., 2004).

2.3.2 Aquaculture wastes and benthos

High accumulation of suspended matter from uneaten food and faeces may affect benthic fauna, sediment chemistry, degradation rate and environmental quality (Clarke & Phillips, 1989; Buryniuk et al., 2006). The deposition of particulate matter from aquaculture net-pens has been identified as the main cause of negative environmental impacts (Vita et al., 2004). The solids emanating from cage farms are particles having size and densities, with a range of settling velocities. These particles are affected by water currents that may vary with depth (Buryniuk et al., 2006). The resulting dispersion may cause settlement well away from the farm and depends mainly on local bathymetry and water movement but usually the highest deposition rates are in the immediate vicinity. On reaching the seabed, these particles may become incorporated into the sediment or resuspended by near-bed currents (Buryniuk et al., 2006).

Through the process of decomposition, oxygen in and above the sediments can become depleted, and under anoxic conditions, gases such as nitrogen, carbon dioxide, methane and hydrogen sulphide can be generated. Also, waste degradation rates could decrease as

anaerobic decomposition generally proceeds at a slower rate than aerobic decomposition does eutrophication (Tovar et al, 2000) which could lead to the formation of chemical species toxic to the fish and can lead to self-pollution and a reduction in the biomass production (Wai et al., 2011).

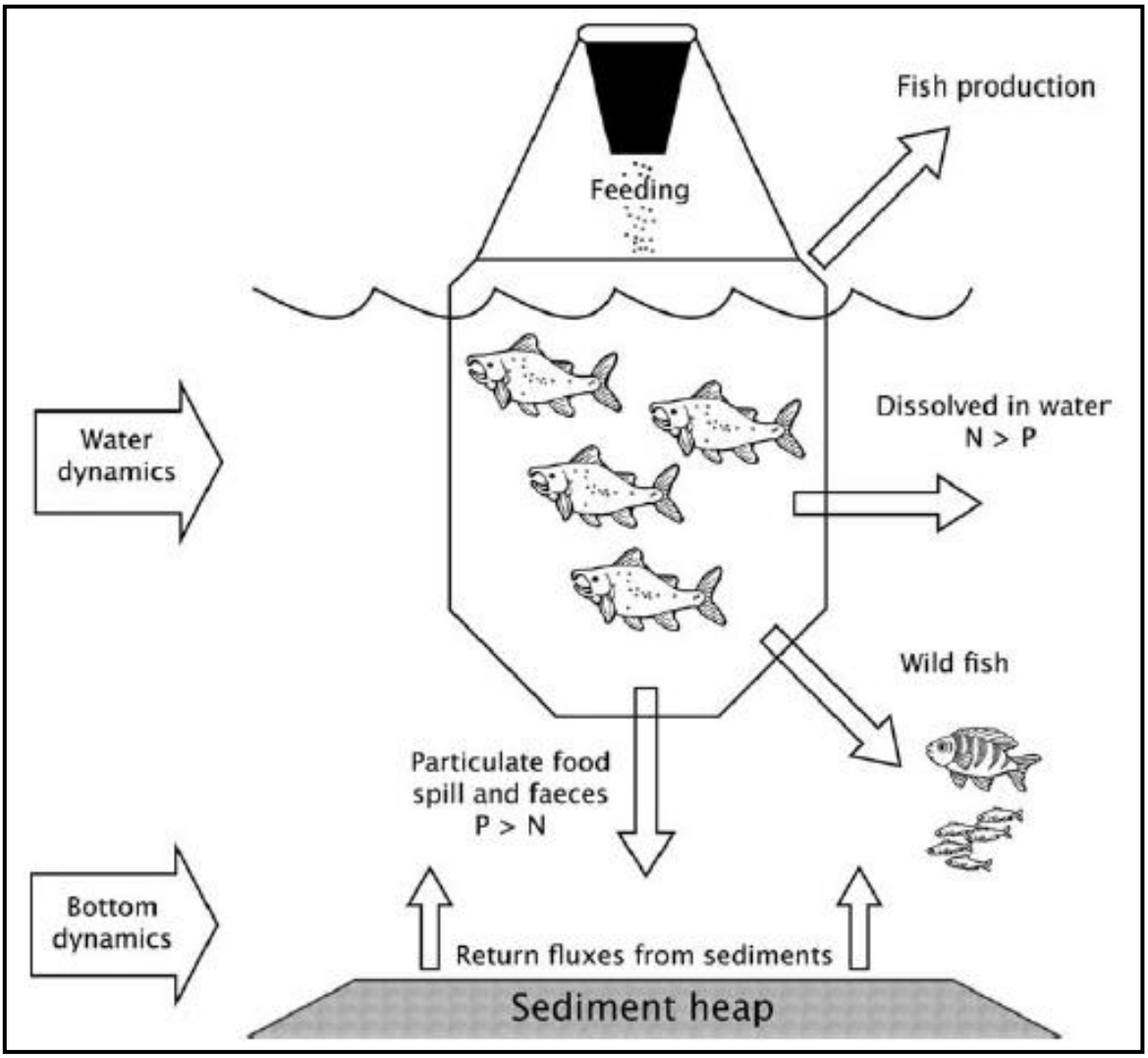


Figure 3. Key components in budgets for phosphorus, nitrogen and suspended particular matter related to emissions from fish cage farms in coastal areas (From Gyllenhammar & Hakanson, 2005)

2.4 Ghost Nutrients and Consumption of Wasted Aquafeeds

Pitta and co-workers (1998) demonstrated in the oligotrophic Aegean Sea (Cephalonia, Ithaki and Sounion Islands) that the benthos was directly affected by sedimentation of organic wastes from farming cages. There was a significant increase in phosphate and ammonium ions but not in phytoplankton biomass which shows the rapid incorporation of the nutrients into the microbial food chain and from there up to larger organisms. It proves that nutrients also called 'ghost nutrient' are discharged in oligotrophic environments by quick transfer via the grazing food chain, from planktonic trophic levels towards higher trophic levels (Pitta et al., 2009). This was also shown in studies of Vita et al (2004); Buryniuk et al (2006); Rountos et al (2012).

Besides, some recent papers pointed out relevant data on the consumption of farmed fish wastes. Dempster and co-workers (2005) showed that planktivorous pelagic species dominate assemblages fishes opportunistically feed upon food pellets lost from cages. In addition, Fernandez-Jover and co-workers (2008) estimated that wild fish around the same fish farms in the western Meditteranean consumed up to 10% of the pellets used at farms. Most of these aggregated wild fish actively consume the lost particulate organic matter (POM), principally in the form of uneaten food pellets and faeces that fall from the cages (Fernandez-Jover et al., 2011).

Objective of the study

This study has focused on the analysis and validation of some selected biomarkers of the interactions between aquaculture cages and the surrounding ecosystem. For this purpose some wild fish communities surrounding aquaculture cages were selected as vectors of determination of the aquaculture effect on the environment, focusing on some fish species belonging to different trophic levels: bogue *Boops boops*, surmullet *Mullus surmuletus*, salema *Salpa salpa* and the yellow mouth barracuda *Sphyraena viridensis*. It is supposed that wastes from aquaculture cages are consumed by wild fish associated to sea-cage farms.

Those aquafeeds coming from sea cages are exogenous nutrients, change the diet of wild fish modifying in this way the physiology, but also the morphology of fishes localized in the immediate vicinity of aquaculture cages. Some morphological and biochemical parameters were considered as biomarkers of the "aquaculture activity" in literature. For instance otolith weight and morphometry, body and scales morphometry and FA's profile.

However, as described in literature, aquaculture activity is not the only human activity that produces inputs of exogenous material to the marine environment that affects those "aquaculture" biomarkers in a significant manner. Thus a parallel study was done on the same fish communities associated to sewage outfalls to compare the effect of other human activities on those biomarkers.

All of the selected biomarkers or biological imprint, considered as useful tools for environmental monitoring of aquatic ecosystems when diffuse pollution is becoming more important (Hansen et al., 2006), are discussed in base of their efficiency to differentiate among wild fish population, fishes associated to aquaculture cages and fishes associated to sewage outfalls.

Material and methods

1. Species studied

1.1 *Salema* Sarpa salpa *(Linnaeus, 1758)*

Es - Salema; Fr - Saupe.

1.1.1 Systematic

Actinopterygii

Perciformes

Sparidae

1.1.2 External description

Present a relatively slender body with 10 golden longitudinal stripes. Show a black spot at the pectoral fin base. Posse 11 to12 dorsal spines, 14 to17 dorsal soft rays, 3 Anal spines, and 13 to15 Anal soft rays (Figure 4) (Muus &Nielsen, 1999).

1.1.3 Distribution

This specie is distributed specially in the Eastern Atlantic: from Congo to South Africa, also present in Bay of Biscay and Strait of Gibraltar to Sierra Leone, including Madeira, the Canary Islands and Cape Verde. Also was reported in the Mediterranean (Riede, 2004)

1.1.4 Habitat

This specie is benthopelagic and oceanodromous that lives in Marine and brackish water. Specially localized in depth range about 5 to 70 m (Bauchot & Hureau, 1990).

1.1.5 Biology

Found especially over rocky substrates and sandy area with algal growth. The juveniles are mainly carnivorous on crustaceans, while the adults are almost exclusively herbivorous and feed on seaweeds. Besides it is a gregarious specie forming size able schools. (Bauchot & Hureau, 1986; Smith & Smith, 1986).

This specie present protandrous hermaphrodite characteristics (Lissia-Frau, 1966, 1968); males are predominantly between 150 mm to 300 mm in length and females from 310 mm to 450 mm. The sex conversion takes place over a wide range of sizes (230–350 mm) (Méndez-Villamil *et* al., 2002); and there hasn't been any external dimorphism character reported that's why the sex determination is based usually only on size (Jadot et al., 2006)

In the last 15 years, this specie has attracted research interest because its role as macro-grazer of seagrass also its toxicity as its consume can cause Ciguatera-like or Caulerpa (Jadot et al., 2006).

Figure 4. Salema *Sarpa salpa* (From Patzner,R.)

Salema has often been observed associated to sea farms, both outside and inside cages, where enter as fry. This species is related mainly to the algae communities associated to floating structures, nets and other structures (Boyra et al., 2004).

1.2 Bogue Boops boops *(Linnaeus, 1758)*

Fr : Bogue, Sp: Boga.

1.2.1 Systematic

Actinopterygii

Perciformes

Sparidae

1.2.2 External description

A fusiform Body, low and slightly compressed, its anterior part is sub-cylindrical in cross section. Large eye, its diameter is greater than snout length. The scales on the top of the head are reaching forward just beyond level of posterior eye margins. Small and oblic mouth. The lips are very thin with incisor-like teeth set in a single row in both jaws.

Dorsal fin with 13-15 spines and 12-16 soft rays. Anal fin with 3 spines and 14-16 pectoral fins short, not reaching to anus. The caudal fin is forked.

Have back bluish to greenish color with silvery or golden reflections in the sides and with 3 to 5 golden longitudinal lines; a small brown spot restricted to pectoral fin axils; and a dark lateral line; light fins (Figure 5) (Bauchot & Hureau 1986; Lleonart et al., 1992).

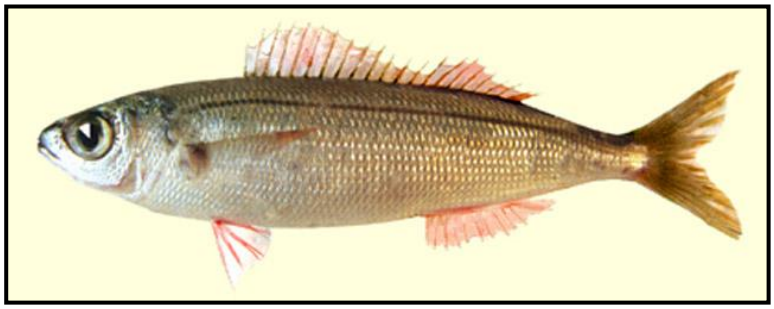


Figure 5. Bogue *Boops boops* (From Pontes, R)

1.2.3 Distribution

Present in the Eastern Atlantic from Norway to Angola including the Canary Islands, Cape Verde, and the Sao Tome Principe Islands. Also common from Bay of Biscay to Gibraltar and found in the Mediterranean and the Black Sea (Bauchot & Hureau, 1986).

1.2.4 Habitat

Found on the shelf or coastal waters, is considered as a demersal as well as semi-pelagic species that lives on all types of bottom (sand, mud, rock, seagrass beds) to 350 m, more abundant in the upper 100 m and sometimes in coastal waters. Moves in aggregations, ascending to the surface mainly at night (Frimodt, 1995).

1.2.5 Biology

Is considered as an omnivorous specie, that feeds mainly on crustaceans, and also planktophagous (Bauchot & Hureau, 1986).

It has been described that Bogue is able to feed wasted pellets from aquaculture sea cages even directly on distributed pellets to compete with reared animals for dry pellets inside sea cages, where they enter as a fry (Dempser et al., 2006; Arechavala-Lopez et al., 2010)

Hermaphroditic, generally protogynous (Frimodt, 1995). The seasonal reproduction varies according to the location, as it occurs in the Eastern Mediterranean between February-April, in the Western Mediterranean between April-May, in the Atlantic it occurs between March-May and finally in the black sea in summer months (Bauchot & Hureau, 1986).

1.3 ***Surmullet*** **Mullus surmulletus** ***(Linnaeus, 1758)***

Fr - Rouget de roche, Sp - Salmonete de roca

1.3.1 Systematic

Actinopterygii

Perciformes

Mullidae

1.3.2 External description

Has a moderately compressed body with a pair of stout barbells under chin, their length is greater than that of pectoral fins; The opercula is without spine; present a less steep snout. The anterior head present a parabolic profile. Has a small villiform teeth in lower jaw and the upper jaw is toothless; teeth are also present on the roof of the mouth (vomer and palatines). Besides, maxilla is at most reaching below the anterior eye margin. First dorsal fin has 7 to 8 spines, the second dorsal fin present I + 8 soft rays with 33 to 37 scales in the lateral line. The body colour is reddish, usually with a darker red longitudinal stripe from the eye to the caudal fin and 3 yellow-brown lines on lower sides (Gharbi, 1980; Renones et al., 1995) (Figure 6).

1.3.3 Distribution

Present in the Eastern Atlantic: From the Western Norway, English Channel (rare in North Sea) to Dakar also in Senegal and the Canary Islands, including the Mediterranean and the Black Sea (Mytilieou et al., 2005)

1.3.4 Habitat

This species affectionate the marine water and is considered as demersal and oceanodromous (Riede, 2004). Distributed in depth range 5-409 m (Mytilineou et al., 2005). Occurs on broken and rough grounds but also found over sand and soft bottoms at depths less than 100 m. It was reported in the studies of Tuya and co-workers 2005& 2006 that large groups of Surmullet were observed associated to aquaculture cages.

1.3.5 Biology

Feeds on benthic organisms such as shrimps and amphipods, polychaetes, mollusks, and benthic fishes. Its spawning periode occurs from May to July. The eggs and larvae are pelagic (Ben-Tuvia, 1990).

Figure 6. Surmullet *Mullus surmuletes* (From Patzner, R.; Stergiou, K.I.)

1.4 Yellowmouth barracuda Sphyraena Viridensis *(Cuvier, 1829)*

Es - Espetón boca amarilla; Fr - Bécune bouche jaune

1.4.1 Systematic

Actinopterygii

Perciformes

Sphyraenidae

1.4.2 External description

Present a slender body, fusiform with a conical hydrodynamical snout. Has a long mouth with low protractile capacity and a prognatic lower jaw. Posed two rows of long canine-like teeth. Has no scale on preoperculum. The upper half of the body present numerous vertical dark bands extending below the lateral line in the anterior part of the flanks (Figure 7) (Barreiros et al., 2002).

1.4.3 Distribution

Present in Eastern Central Atlantic including Cape Verde and the Canary Islands and in the Azores Islands. Also reported in the eastern Mediterranean specifically in Lebanon. Its exact distribution and abundance are unknown because most published records do not separate it from *Sphyraena sphyraena* (Barreiros et al., 2002).

1.4.4 Habitat

This specie affectionate the marine water usually present at depth 50 m up to 100m (De Sylva, 1990). In the study of Boyra and co-workers 2004, *Sphyraena viridensis* were observed associated to aquaculture cages in Gran Canaria and were found abundant.

1.4.5 Biology

Feeds on cephalopods, crustaceans and fishes (Ben-Tuvia, 1986).

Figure 7. Yellowmouth barracuda *Sphyraena viridensis* (From y Patzner, R.)

2. Sampling zones: Field collection of studied species

The Canary Islands are part of the marine ecosystem of the canary current which is distributed among the Atlantic African North occidental, this current have the characteristics of being very soft in the coastal part and reach higher speeds in the archipelago of Canarias, where warm waters are being created. The canaries marine waters are oligotrophics with a great mesoscalar variability in primary production, the waters are vertically mixed (African up-welling) and transport nutrients to the costal zones. There is also some eutrophical areas in the canaries zones of transition (Oceana, 2010).

In order to obtain samples from different zones (Figure 8) some sampling areas were determined in Gran Canaria Island. Two aquaculture-related zones have been selected at the Eastern coast of Gran Canaria:

- ✓ Melenara, Taliarte
- ✓ Castillo del Romeral

These two zones are specialized in marine aquaculture, rearing mainly European sea bass *Dicentrarchus labrax* (Linnaeus 1758) and Gilthead sea bream *Sparus aurata* (Linnaeus, 1758).

To compare samples from aquaculture influences with samples submitted to other human influences or with samples without human influences, samples have been collected from two different zones (Table I):

- ✓ Wild zones: 4 sampling points have been selected from virgin zones that were not submitted to any kind of pollution or anthropical impact.
- ✓ Urban zones: 4 sampling points have been selected near harbors and emissaries, which were zones with organic waste-waters.

In each sampling point, at least 10 animals were captured (Table II). All animals were captured by direct fishing, from both technicians and local fishermen using fishnets (Bogue, Salema) fishing rods (Bogue, Surmullets) or using fishing rifle while diving (Yellow mouth barracuda).

All the fish collected were immediately frozen at -20°C, until they were processed.

Table I. Geographic position of the sampling sites and their type

Sample Zone Name	GPS coordinates	Type of Zone
Zone I	28° 09' 312" N 15° 24' 184" W	Urban
Zone II	27° 50' 747" N 15° 23' 705" W	Urban
Zone III	28° 02' 810" N 15° 22' 455" W	Urban
Zone IV	28° 09' 516" N 15° 42' 530" W	Wild
Zone V	27° 51' 14,78" N 15° 23' 11,28" O	Urban
Zone VI	28° 09' 04,76" N 15° 42' 07,75" O	Wild
Zone VII	28° 10' 06,32" N 15° 42' 22,58" O	Wild
Zone VIII	27° 46' 48,26" N 15° 43' 01,26" O	Wild
Aquaculture 1	27° 47' 42,66" N 15° 26' 46,71" O	Aquaculture
Aquaculture 2	27° 58' 45,81" N 15° 22' 19,91" O	Aquaculture

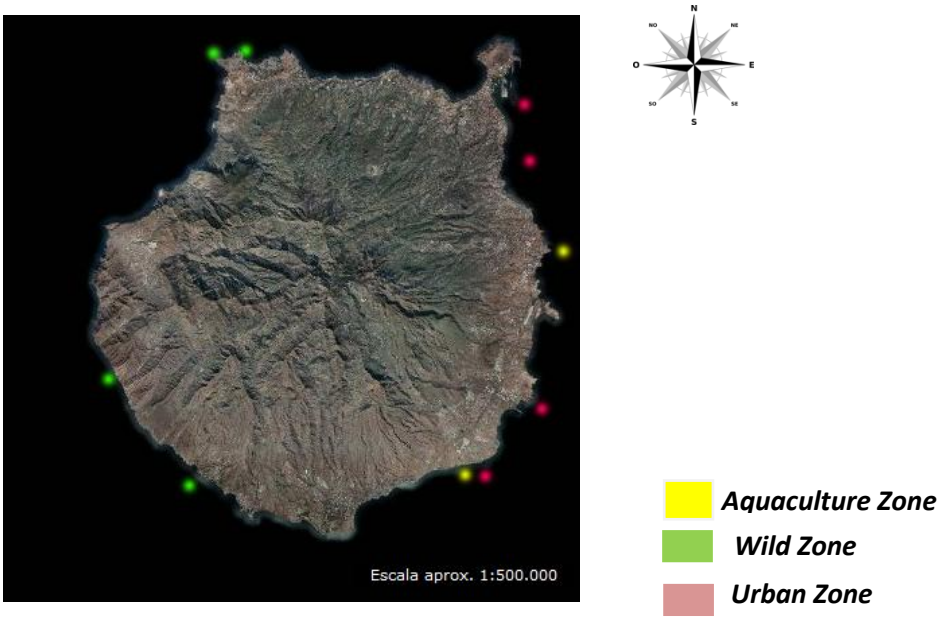


Figure 8. Gran Canaria map localizing the sample areas (From Grafcan) [3].

Table II. Total of samples from each specie

Especies	**Bogue**	**Salemas**	**Surmullet**	**Yellowmouth baracuda**
Total	118	78	58	23
Aquaculture	40	27	14	8
Urban zone	38	11	14	10
Wild zones	40	40	30	5

3. Fish process

3.1 Images for morphological studies

To start the fish process, frozen fishes were de-frozen to ambient temperature 12 hours before the starting of the process (Figure 9).

Figure 9. De-frozen of the Salemas 12h before starting the process

Following the protocol used by Grabana & Saborido-Rey [3] pointers are set in the skin (Figure 10) of the fish body, to help identify the landmarks that will be used in the photos. Those are anatomical points with biological label which means that those landmarks were selected principally to define the outlines of fish's body but also because they are easily recognizable in all fishes from the same species.

a) **b)**

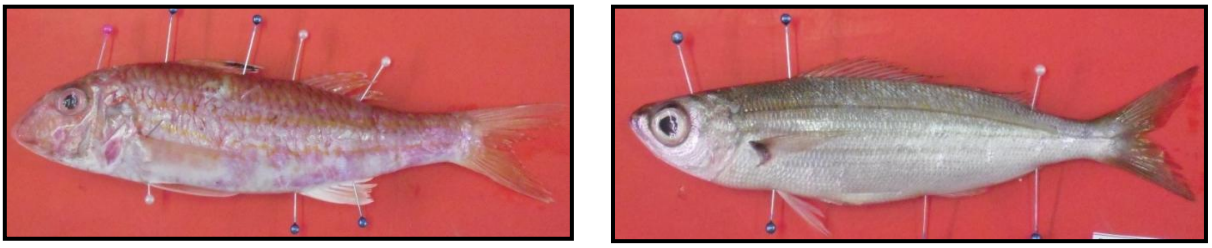


Figure10. Using pointers to localize key point in the Body of the Surmullet (a) and Bogue (b)

Then a series of photos is taken of each individual with a digital camera (Casio Exilium EX-Z35). After this, total length and the total weigh of the individual were recorded.

3.2 Extraction of the otoliths and the scales

The otoliths were removed in pairs in case of any possible damage or loss during the measuring process (Figure 11). The sagittal otolith pairs were cleaned and stored in dry conditions and measurements were always obtained from undamaged otoliths. Moreover the weight of the right Sagittae (*Ow*) was taken to the nearest 0.01mg.

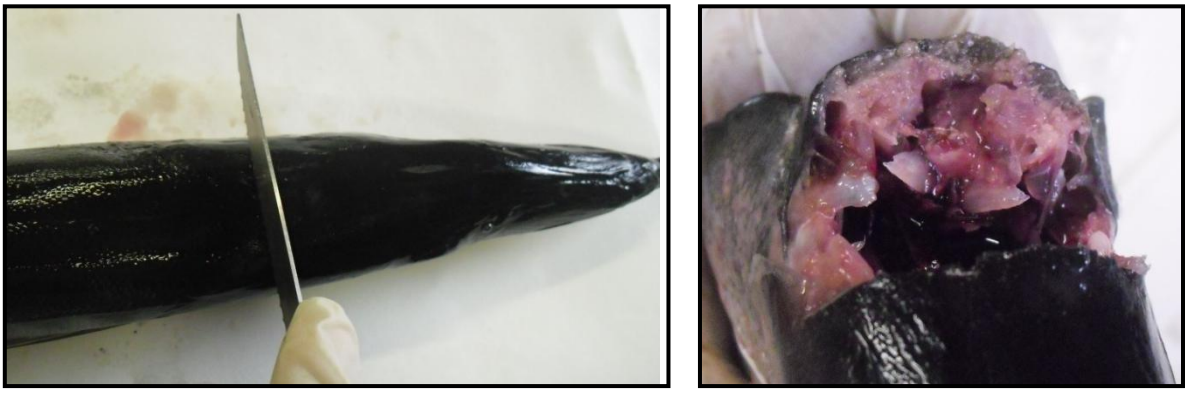


Figure 11. Removing the Sagittal otolith pair from *Yellowmouth barracuda*

Also, the scales were taken (Figure 12) using a clamp from the lateral part near the fork and in the upper range of the lateral line.

a) **b)**

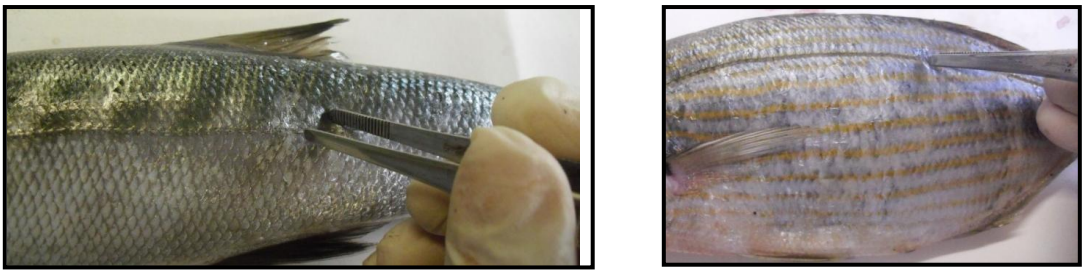


Figure 12. Extracting scales from Yellowmouth barracuda(a) and Salema (b)

3.3 Otoliths and Scale Image Digitalizing

3.3.1 Digitalizing of the otoliths

Following the method of Tusset and Co-workers (2003), Pictures of the otoliths was digitized using a digital camera under a binocular microscope (Leica) with the most convenient magnification in each case and connected to a computer (Figure 13)

For the otolith, the image was taken of the internal side (medial or proximal) of the otolith as this side presents the sulcus acusticus (a groove along the surface of the sagitta). To obtain a good representation of the sagitta contour, the image must be well contrasted with homogeneous black background.

a) **b)**

Figure13. Otolith digitized of the Bogue(a) and the Surmullet (b)

3.3.2 Digitalizing of scales

The protocol described by Clifford and co-workers (2011) have been followed to prepare the scales for the image analysis: The scales have been soacked in distilled water for a short period of time. When the scales became flexible, they were mounted on a pair of laboratory glass slides. To keep the scales moist, care was taken. This was done to make sure that the scales do not curl or fracture prior to positioning the cover slide. Pictures of

scales were digitized using a digital camera under a binocular microscope (Leica) with the most convenient magnification in each case and connected to a computer (Figure 14).

a) **b)**

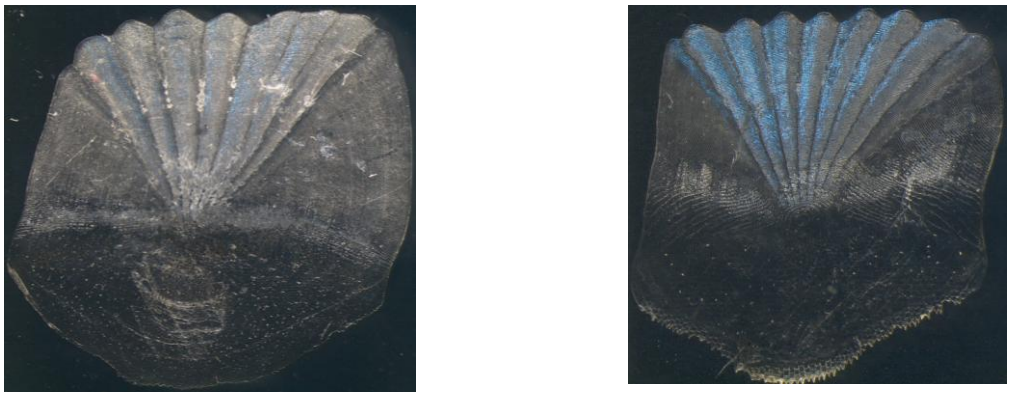


Figure 14. Scales digitized of Salema (a) and Bogue (b)

3.4 Inter-zones variability in the otolith weight

To check the homogeneity of total length between all the groups, an univariate analysis was performed using the software SPSS 15.0.When the left otolith was missing or broken, its weight was replaced with the weight of the right one. It was reported that there wasnt' any statistical difference between right and left otolith weight (Cardinal et al., 2004; Mc Dougall, 2004)

$$Ow = a\,Tl^{b}$$

$Ow = Otolith\ weight$

$Tl = Total\ lenght$

$a = allometric\ constant$

$b = allometric\ constant$

To avoid allometry effects of the fish length in the otolith weight, this variable was normalized taking into account the allometric relationship (Lombarte & Lleonart, 1993).

$$Ow' = Ow\left(\frac{\overline{Tl}}{Tli}\right)^{b}$$

$Ow' = Otolith\ weight\ ponderated$

$$\overline{Tl} = \sum Tli$$

$Tli = Total\ lenght\ for\ the\ individu\ (i)$

After performing segmentation according to the sampling zone, the variable (b) was analyzed by a regression using a curvilinear estimation with the linear and potential model. The otoliths were always represented with the respective dorsal margin on the top of the image and anterior (rostral) region to the right.

3.5 Biochemical and fatty acid content of fish fillet: sample collection

After the collection of scales and the otolith, the animals were eviscerated and weighted to record the eviscerated weight. The livers were then obtained by dissection. After that, a part of the anterior dorsal white muscle from each fish was removed, the skin was removed out and the fillets were put in plastic bags without air, for a better conservation. Finally fillet and liver samples were frozen at -20°C for biochemical analysis (Figure 15).

Figure 15. Removing the fillet for biochemical analysis

4. Selected biomarkers

4.1 Generalities

It has been reported that there is a clear difference in body morphology between wild and reared fishes from the same specie (Vay et al., 2007). These differences can be due to genetic adaptation or also from the impact of the environment. Selection pressure on heritable traits governing shape could differ among fishes growing in different environments, leading to greater survival of some genotypes in different habitats (Von Cramon- Taubdel et al., 2005).

In other words, it is expected to find morphological difference between the same species of fish living in different environments as it was reported inter-specifically for wild and reared fishes (Hard et al., 2000; Arechavala-Lopez et al., 2011; Rogdakis et al., 2011) or for the same specie leaving in different biotopes as it was reported in the study of Traina and co-workers (2011). These authors adopted a morphometrical approach to discriminate among anchovy populations collected from Sicily, Tunisia and the Adriatic Sea and

detected two anchovy sub-population between the Sicilian and the Adriatic population connected by migration of a small number of individuals.

4.2 Geometric Morphometric Method (GMM)

The morphology has been traditionally studied using classic morphometric parameters (Jaiswar et al., 2004). This method determinate morphological aspect of fishes and could be very useful for studies of first record and studies that differentiate between populations (Rogdakis et al., 2011). However, one of the major problems in traditional morphometrics is that linear distance measurements are usually highly correlated with size (Brokstein et al., 1985). To avoid this type of problems, we choose the Geometric Morphometric Method, described by (Bookstein, 1991) to study all the morphometric parameters.

GMM analyzes the relative positions of anatomical landmarks and sets of points used to approximate curves and surfaces to quantify size and shape (Jensen, 2003). Geometric morphometric methodology implies multivariate analysis of landmark coordinates located by following certain rules on the surface of a morphological object: The specimen is described in the space by a set of landmarks X and Y coordinates which are homologous and can be recovered unambiguously from a specimen to another (Bookstein, 1991).

4.2.1 Choosing Landmarks

The landmarks chosen are related to functionally relevant aspects of the form of the fish. This step is crucial and requires a fine observation of the body shape with the use of outline methods. After an external observation of the body of different species, we localized points that could be susceptible to distinguish when they change that could be easily identified (Ambrisio et al., 2008).

4.2.2 Software applications

After chosing the landmarks (Figure 16), they were digitalized using the software **TPS Dig software**[2] (Rohlf, 2006) coordinates were superimposed by means of generalized Procustes Analysis (GPA), this method preserves all information about shape (scale, position, and orientation) (Rholf & Slice., 1990).

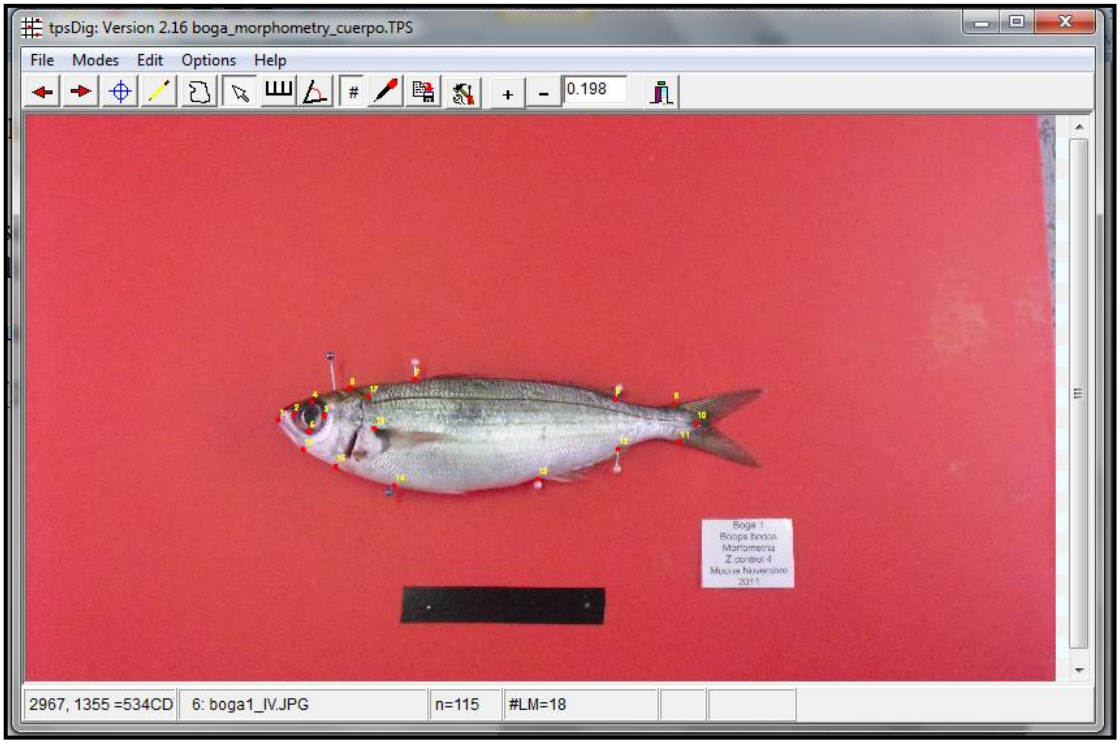


Figure 16. Choosing Landmarks to study morphometry of the Bogue

Then, the software **TPS Relw software** [2] (Rohlf, 2006) was used to effectuate a relative warp analysis (similar to a principal component analysis) on the covariance matrix derived from the partial warp scores. This analysis was used to describe the main shape variation. The centroid size (SC), defined as the square root of the summed square distance of all landmarks about their centroid was calculated as a measure of overall scale size (Ambrioso et al., 2008).

To explore the differences among the samples, a Relative Warp Analysis (RWA) was conducted. The relative warps are computed to summarize the variation among the specimens with respect to their partial warp scores is used to quantify changes in shape and pattern of morphometric variation within and among groups, when each individual is considered as fitting into a *consensus* configuration (Cadrin, 2000)

A clasdogram of Procrutes morphological distances was performed with the complete linkage algorithm to observe distances among the different samples (Bookstein., 1991). The splines (deformation grids) of the extremes of each RWA axis and clastrograms were extracted using the TPS Relw (Rohlf., 2006).

4.2.3 Body morphology

4.2.3.1 Processing the individuals

The method followed was described by Ambrisio and co-workers (2008). After an external observation of the body of different species, points susceptible to distinguish when they change and could be easily identified were localised .Those points must have a biological meaning and they are called Landmaks, in addition we chose other points that could help us adding more information about the shape of the animals.

On the photograph of each individual, series of landmarks are chosen (Figure 17)

- ✓ 21 Landmarks for the Salema *Sarpa salpa*
- ✓ 18 Landmarks for The Bogue *Boops boops*
- ✓ 19 Landmarks for the Surmullet *Mullus surmuletus*
- ✓ 20 Landmarks for the yellowmouth barracuda *Sphyraena sphyraena*

After, the softwares TPS Dig and TPS Relw software are used (Rohlf, 2006).

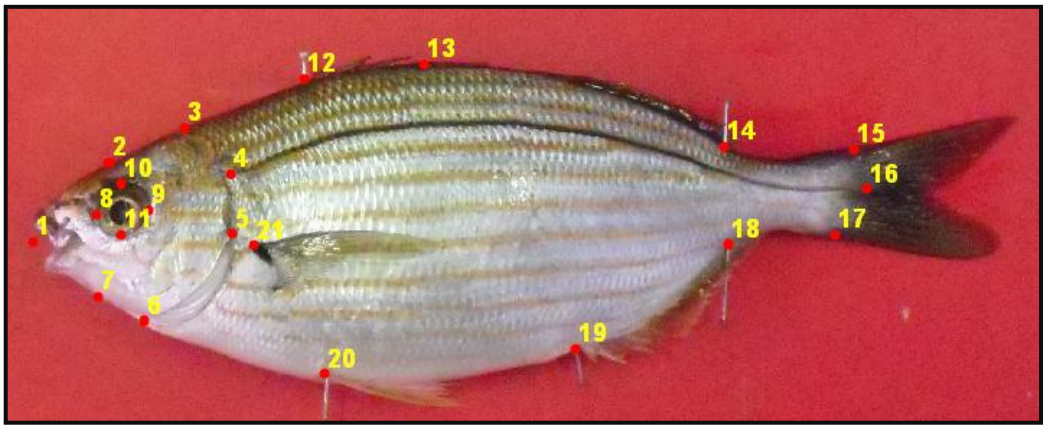


Figure 17. Example of Landmarks chosen on Salema's body

4.2.4 Scales morphology

The method described by Ibañez and co-worker (2007) have been followed. Preliminary visual assessment was used to identify potential landmarks on the scales. The landmarks chosen were located on key features of the scale (Figure 18) that are common to all scales of the species or variants examined , this ensures that in subsequent interpretation of results, variations in particular landmarks can be related back to shared features of shape.

- ✓ Landmarks 1 and 3 are respectively the ventro- and dorso-vertral tips of the anterior portion of the scale

- ✓ Landmark 2 is in the center of the anterior edge of the scale
- ✓ Landmarks 4 and 6 are located at the boundary between the anterior area with circuli and the posterior area covered by Cteni
- ✓ Landmark 5 is in the focus of the scale
- ✓ Landmark 7is located at the tip of the posterior portion of the scale.

The configuration of landmarks coordinates of body and scales were scaled, translated and rotated using generalized procrustes analysis (Cadrin, 2000).

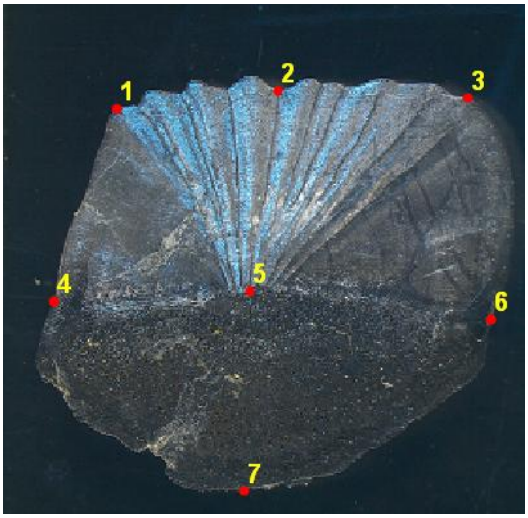


Figure 18. Landmarks chosen were located on key features of the scale of Salema

Scales statistic analysis was performed by the software SPSS 15.0. The multivariate Box M test for homogeneity of variances/covariance is particularly sensitive to deviations from multivariate normality Box's M tests the null hypothesis that the covariance matrices do not differ between groups formed by the dependent. Then they were submitted to a discriminate analysis with the cross validation option:

Cross-validation is often used to estimate the generalization ability of a statistical classifier, the available data are divided into k disjoint sets; k models are then trained, each on a different combination of k – 1 partition and tested on the remaining partition. The k-fold cross-validation estimate of a given performance statistic is then simply the mean of that statistic evaluated for each of the k models over the corresponding test partitions of the data. Cross-validation thus makes good use of the available data as each pattern used is used both as training and test data. Cross-validation is therefore especially useful where the amount of available data is insufficient to form the usual training, validation and test

partitions required for split-sample training, each of which adequately represents the true distribution of patterns belonging to each class. The most extreme form of cross-validation, where k is equal to the number of training patterns is known as leave-one-out cross-validation, and has been widely studied due to its mathematical simplicity. A property of the leave-one-out cross-validation estimator, often cited as being highly attractive for the purposes of model selection is that it provides an almost unbiased estimate of the generalization ability of a classifier (Cawley & Talbot., 2003).

To test the sample's homogeneity in terms of size, a multivariate test for a general linear model predicting shape variation as a function of an independent size variable (Computed as centroid size). Wilks' lambda is a direct measure of the proportion of variance in the combination of dependent variables that is unaccounted by the independent variable, and indicates the significance of the discriminant function. To test whether there are differences between the means of identified groups of subjects on a combination of dependent variables (Crichton, 2000).

4.2.5 Otolith morphology analysis

The Wavelet Transform (WT) is based on expanding the contour into a family of functions obtained as the dilations and translations of a unique function known as a mother wavelet (Mallat, 1991). These functions describe both in space and wave number, the most prominent features of the curve. The signals of wavelets have different amplitudes, hence small (low) wave-numbers (frequencies) are associated with a smoothly varying contour, while large wave-numbers are associated with variation on a small spatial scale (Parisi-Baradad et al. 2005, 2010). To obtain the otolith contour, a total of 512 cartesian coordinates on each of the orthogonal projections, which is a perpendicular image of an object (Figure 19) (Rholf, 1985), were extracted using Age & Shape program (Infaimon SL, Spain) software Image Pro Plus. The wavelets five were selected as the representatives of the otolith contour.

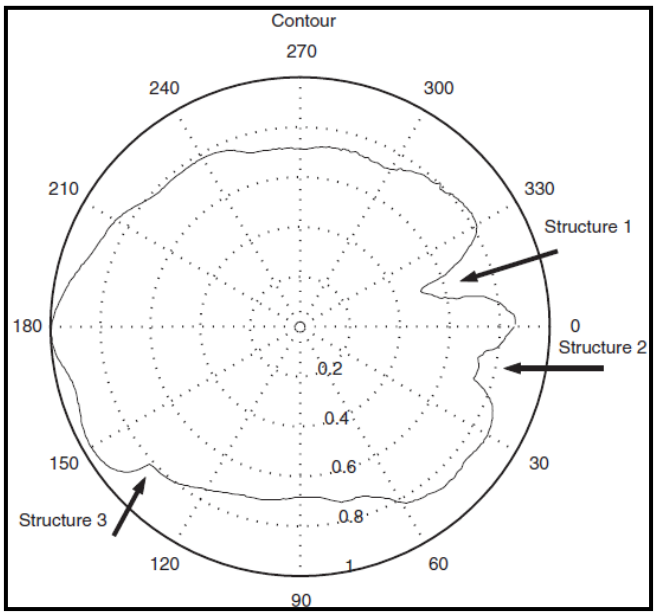


Figure 19. Planar polar representation of the otolith contour, the contour is resolved by 512 points sampled representing three most important structure of the otolith contour (From Parisi baradad , 2005).

Concretely, the method used is based on random projections introduced by Cuesta-Albertos & Febrero-Bande (2010) that works as follows: (i) random projections are used to transform functional data into univariate data, (ii) the anova problem is then solved in this simple one-dimensional scenario, and (iii) conclusions for the functional data are obtained by collecting the information from several projections.

Finally, to test the significance of the p value both the Bonferroni method and the False Discovery Rate method have been used for the analysis of the otolith of Bogue from the three groups.

4.3 Biochemical analysis

4.3.1 Sampling process

9 individual of Salemas were selected from the aquaculture, wild and urban groups as samples for the biochemical analysis.

4.3.2 Biochimical analysis

After individual tissue homogenization, moisture content was determined after drying at 105 °C to constant weight. The FA composition of the total lipid fraction was determined after fat extraction following the method of Folch, Lees & Stanley (1957), with a mixture of chloroform and methanol (1:1 proportion for the first extraction and 2:1proportion for the second one). Fatty acids from total lipids (stored under nitrogen atmosphere at−80 °C) were prepared by trans-methylation as described by Christie (1982) and separated by gas chromatography under the conditions described by Izquierdo et al. (1990) being quantified by flame ionizator detector (FID) and identified in comparison to external standards of CLA isomers (Sigma-Aldrich and Matreya, LLC.) All analyses were conducted in triplicate.

4.3.3 Statistical analysis

Means and standard deviations (SD) were calculated for each parameter measured. Data were submitted to a one-way analysis of variance (ANOVA) to analyze the different data reported for each group of Salema. The program SPSS 15.0 was used. When F values showed significance, individual means were compared using Tukey's test for multiple means comparison. Significant differences were considered for $P<0.05$.

Also, the PRIMER statistical program was used to perform a Principal components analysis (PCA) which is considered as an ordination method. Variables that had more influence on similarities within groups and dissimilarities among the groups of Salemas were calculated using the SIMPER (similarity percentages) procedure (Clarke, 1993).

Results

1. Otolith weight

The curvilinear regression performed to estimate the allometric coefficient (b) of fishes from different groups aquaculture, wild and urban are presented in Table III.

Table III. Allometric coefficient (b) for selected species

b / Species	Aquaculture	Wild	Urban
Surmullet	1,091	2.311	-
Bogue	1,325	1.295	1.732
Salema	1.484	1.891	2.155
Yellowmouth baracuda	1.994	2.222	-

This allometric coefficient in Salemas coming from the aquaculture zone have the lowest weight of otolith with respect to the ones coming from wild and urban zones. The same observation can be made about the allometric coefficient (b) for the Surmullet as the otolith weight of samples coming from the aquaculture zones were lighter than those from wild ones.

Also the same tendency is observed for the Yellowmouth bararcuda as the Sagittae weight of species from aquaculture zones is lighter than those from wild ones.

The only exception on the results for the allometric coefficent (b) parameter is found in the Bogue, where we observed that the otolith of individuals from aquaculture zones are slightly heavier than those from wild species, whereas the otolith form urban zone are the heaviest ones. Although, this result may arise due to the fact that most otoliths from Bogue were damaged, this may have affected the otolith weight repartition among the 3 different sampling zones.

2. Otolith morphometry

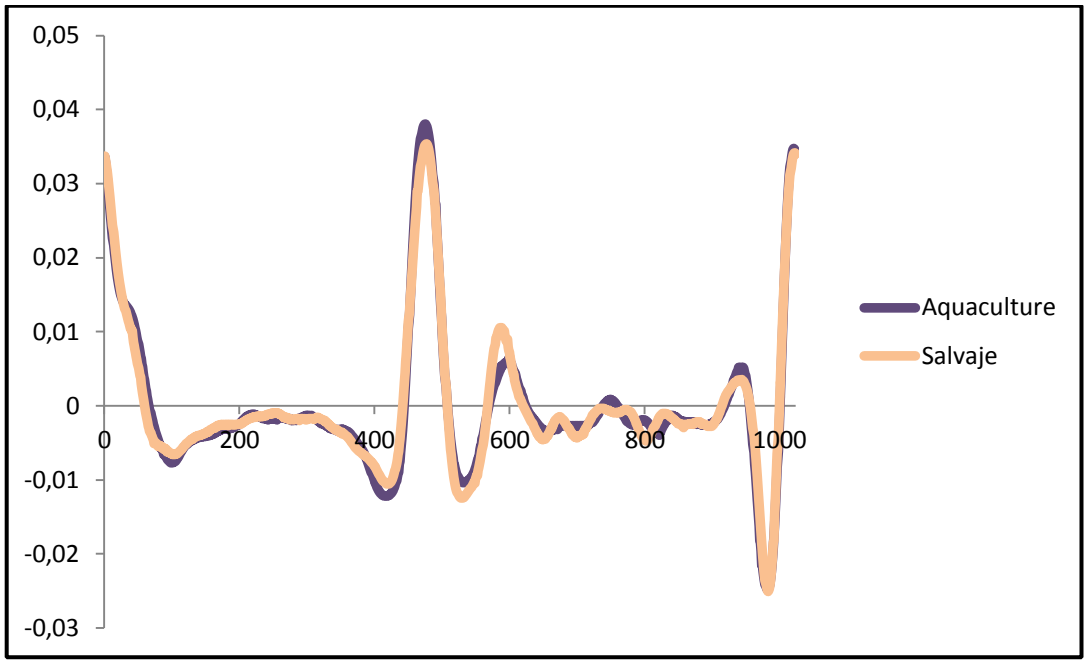


Figure 20. Wavelet transform (WT) of the Salema otolith

The figure 20 is a representation the 5th wavelet of Salemas from aquaculture and wild groups. The peak of the wavelet transform (WT) indicates the main visual cues of the shape. From the graphical, it appears that the 5th wavelet present similar peaks either for aquaculture or wild salemas. Then we can say that there is no shape difference in the otolith of these species.

The same observation has been made for the Bogue, where the representation of the 5th wavelet from aquaculture, wild and urban groups presented also similar peaks of each one of these groups. It was observed that there was no shape difference in the otolith belonging to aquaculture, wild and urban groups of Bogue. The P-value test effectuated on WT on the otolith of Bogue was reported in the following table (Table IV).

Table IV. Results of P-value obtained with Bonferroni method and the False Discovery Rate method

p-value for Bonferroni method	p-value for False Discovery Rate method
RP2 0.65790	RP2 0.65790
RP5 0.81403	RP5 0.58616
RP15 1.00000	RP15 0.75364
RP30 1.00000	RP30 0.78102

For both of these tests there were no significant difference between species (P<0.05). This also confirms the results obtained by the WT representation.

3. Body morphometry

The analysis of the body morphology was done using the discrimination method, which is performed on the Weight matrix (rows are specimens, cols are x,y pairs) generated from the warps by the software Tps Dig.

3.1 Salema body morphometry

In the case of body morphometry of Salema, the program generated 38 relative warps. The Figure 21 represent the dispersion points of relative warps represented for the axes X=1 and Y=2.

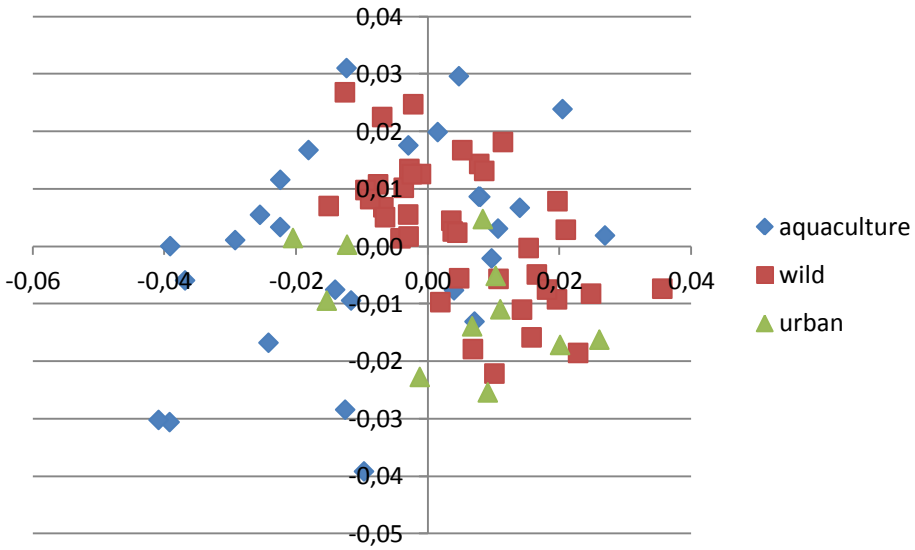


Figure 21. Relative warps ordination plot of Salemas Body .Axes (X=1;Y=2)

All the classification test of relative warps analysis were made using discrimination test of the 10 first warps, because they concede the most important variation within each group. The cross validation reveled a correct classification to the 77.9%.

With this classification system, Salema from aquaculture zone were correctly classified as aquaculture species to the 77.8%, equally classified as urban and wild groups at 11.1%. Salemas from wild group were correctly classified to the 74.4%, classified with urban group to the 10.3% and classified as aquaculture to the 15.4 %. And finally, Salemas from urban group were correctly classified to the 72.7%, classified with aquaculture group to the 18.2% and classified with the wild group to the 9.1%.

The Discriminant Canonical Analysis is showed in Figure 22, as the geometric morphometric method, of capturing information about curves or outlines of organismal structures may be used in conjunction with canonical variates analysis (CVA) to assign specimens to groups or populations based on their shapes (Sheets et al., 2001).

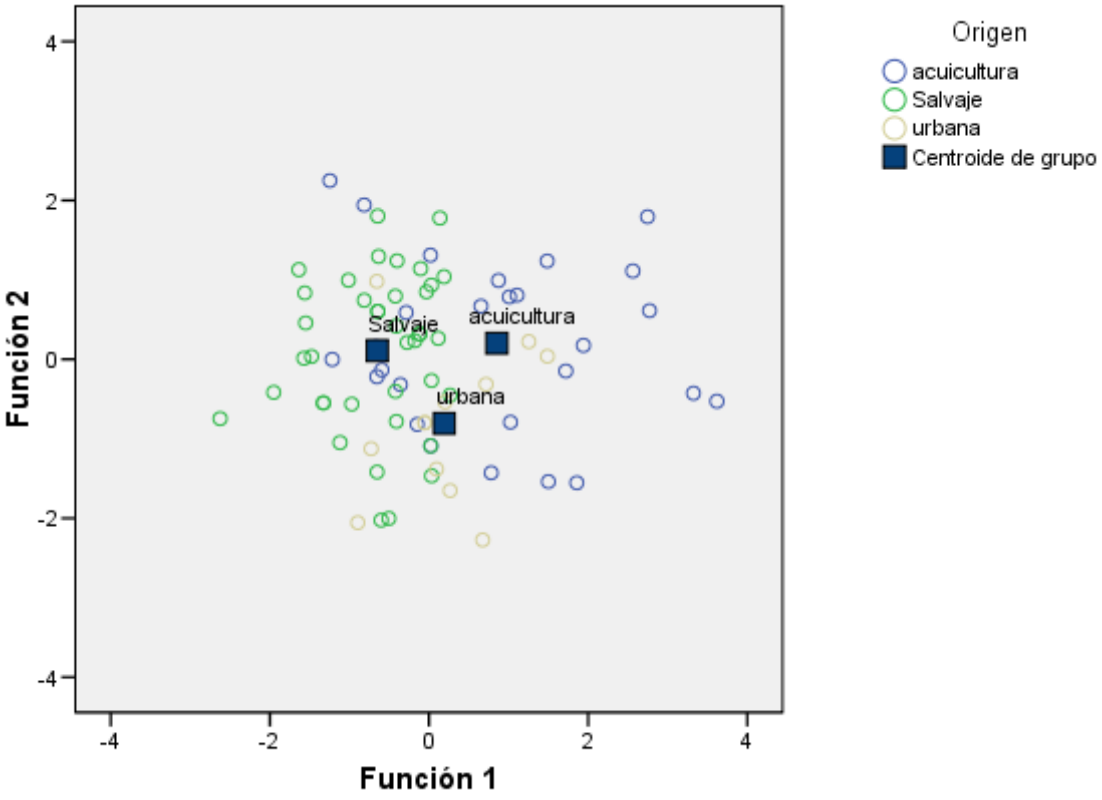
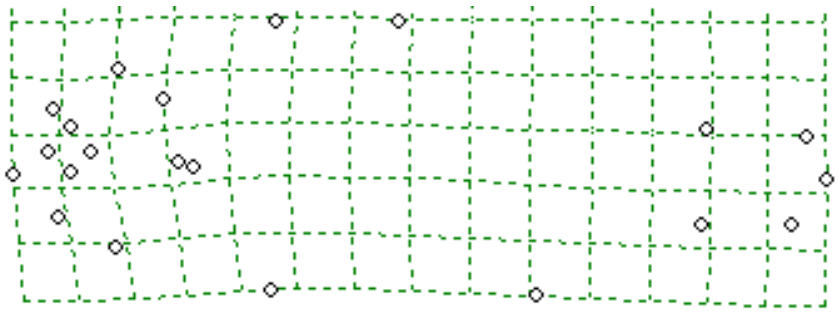


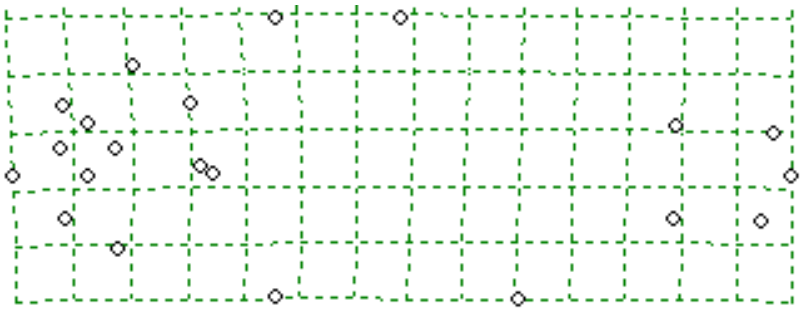
Figure 22. Landmark-based method : The Discriminant Canonical Analysis of the overall body shape variation of Salema along the first two canonical axes

Canonical variates analysis based on both methods separate the group of species into three clouds of points. Each group is agglomerated around a centriode. The aquaculture, urban and wild groups are clearly separated, with the urban cluster in the middle of the two other ones. The contrast of the function 2 to the function 1equal to lambda of Wilks= 0.41

a)



b)



c)

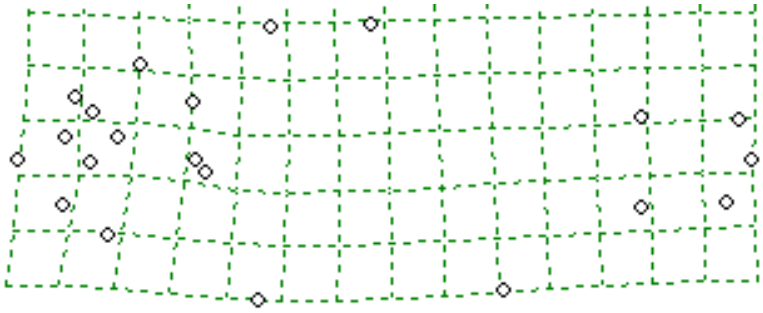


Figure 23. Deformation grid of Salemas body from a) aquaculture zone b) wild zone c) and urban zone

Comparing the body morphometry of the Salemas from the three groups (aquaculture, wild and urban) (Figure 23) there are some differences in the head area. Therefore, to refine the results we concentrated on the cephalic region by increasing numbers of landmarks and semi-landmarks in that region. Consequently, 13 Landmarks (Figure 24) have been selected in the head region to focus on the morphometrical differences localized in cephalic region.

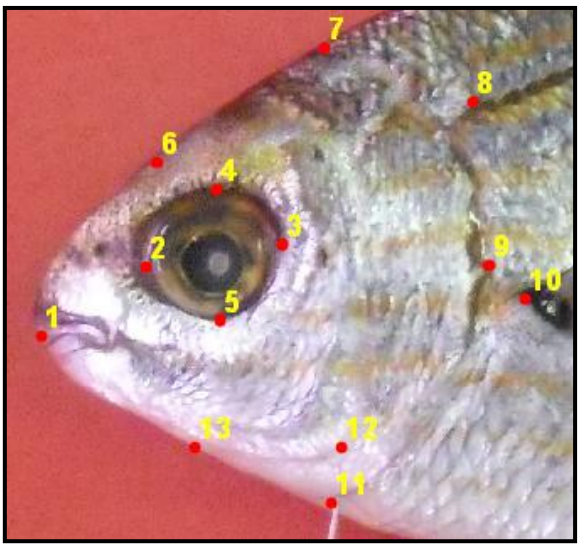


Figure 24. Landmark addition in the head region of Salemas

The warps from the Salema's cephalic region plotted via cross-validation analysis were correctly classified at 61%. Salemas from aquaculture groups were correctly classified to the 63% and equally classified with the Salemas from urban group to the 37%. There is no similarity in the classification between aquaculture and wild groups.

Salemas from wild groups are correctly classified to the 61.5% and equally classified with urban groups to the 33.3%, while the classification between wild and aquaculture groups are estimated to 5.1%.

Salemas from urban groups were correctly classified to the 27.3% and equally classified with wild groups to the 36.4% and also with aquaculture groups to the 36.4%.

Those results on the head region follow the same tendency observed earlier with morphometry of the Salema's body, and confirm that the major part of the differences is localized in the cephalic region.

3.2 Bogue body morphometry

In the case of the body morphometry of Bogue, the program generated 32 relative warps. The figure 25 represent the dispersion points of relative warps represented for the axe X=1 and Y=2.

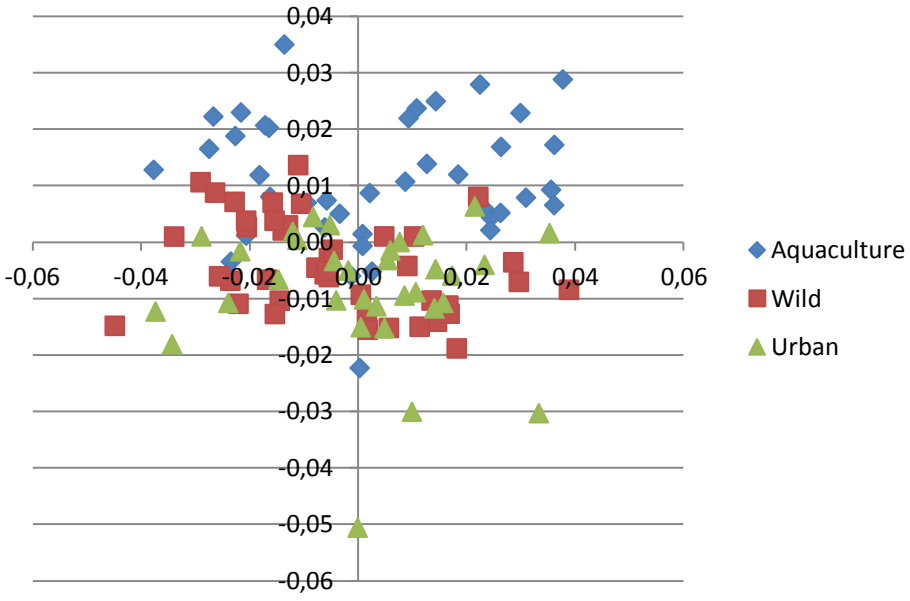


Figure 25. Relative warps ordination plot of Bogue's body, Axes (X=1;Y=2)

The relative warps from Bogue's body were plotted via cross-validation analysis and were correctly classified at 64.3%.

The aquaculture groups were correctly classified to the 72.5%, then 20% as wild while 7.5% of those Bogue were classified as urban ones. Wild group of Bogue were correctly classified to the 52.4% and assimilated to the aquaculture groups to the 31% and 16.7% of them were classified as wild ones.

The urban groups of Bogue were correctly classified to the 63%, while 33% of them were assimilated to wild groups and only 3% were assimilated to aquaculture groups.

The results of the discriminant canonical analyses are plotted in (Figure 26). The canonical discrimination function showed a clear separation between the 3 groups: aquaculture, wild and urban. Each group was represented by its centroid. In this case, urban and wild groups seem to be more closely related, whereas the aquaculture group seems to be separated to two other ones.

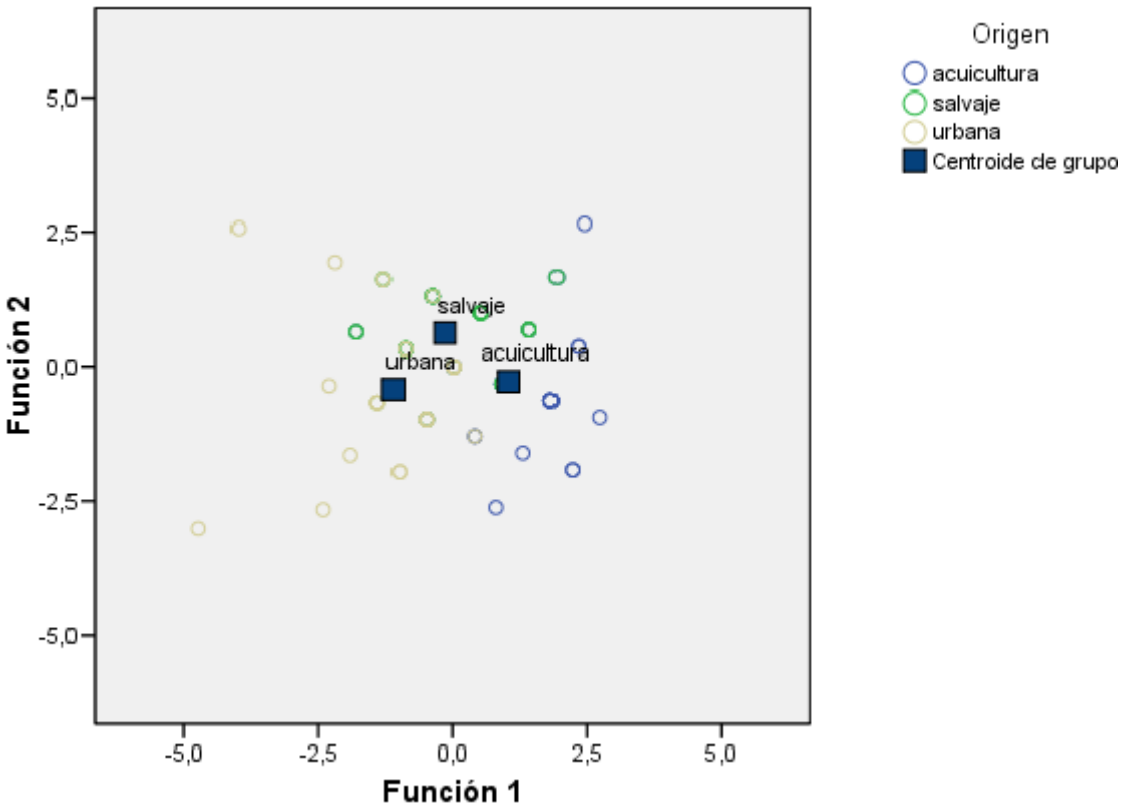


Figure 26. Landmark-based method : The Discriminant Canonical Analysis of the overall body shape variation of Bogue along the first two canonical axes

The canonical discriminant function clearly separated the centroid of aquaculture group from the wild and the urban ones**.** The contrast of the function 2 to the function 1 was explained by lambda of wilks= 0.436.

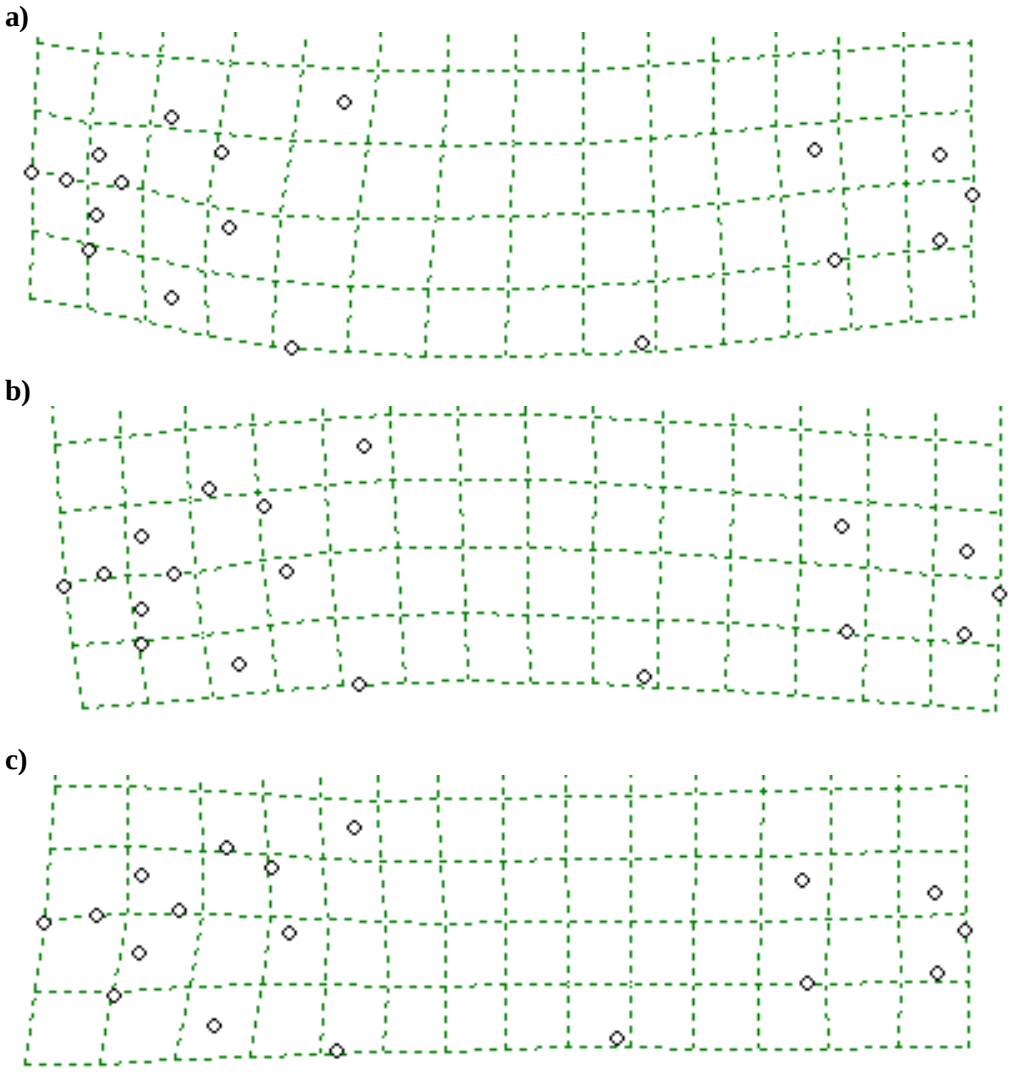


Figure 27. Deformation Grid of the Bogue's body from a) aquaculture, b) wild and c) urban groups

The observation of morphometry of Bogue's from the generated deformation grid in the 3 different groups pointed out major differences in the cephalic region (Figure 27). So we concentrated on that region adding 12 landmarks and semi-landmarks to help us localize the origin of this difference (Figure 28).

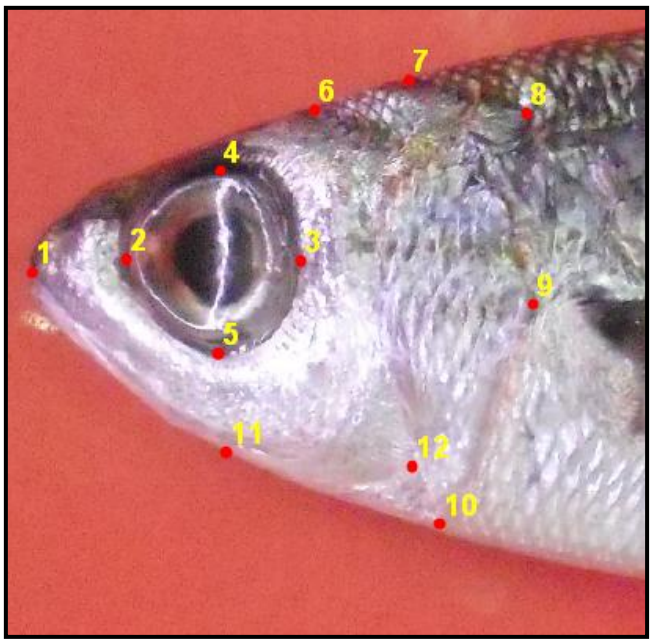


Figure 28. Landmark addition in the cephalic region of Bogue

The cross validation test plotted on relative warps from the head of Bogue made a correct classification to the 71.8%. The aquaculture group of Bogue were correctly classified to the 77.5% and showed a similarity with urban groups estimated to the 15% with only 7.5%.with the wild group. The urban groups were correctly classified to the 52.9%, whereas 32.4% were assimilated to wild ones and 14.5% were assimilated to aquaculture group. Finally, the wild group was correctly classified to the 62.8%, assimilated to the urban ones with estimation of 30.2% and 7% to the aquaculture group.

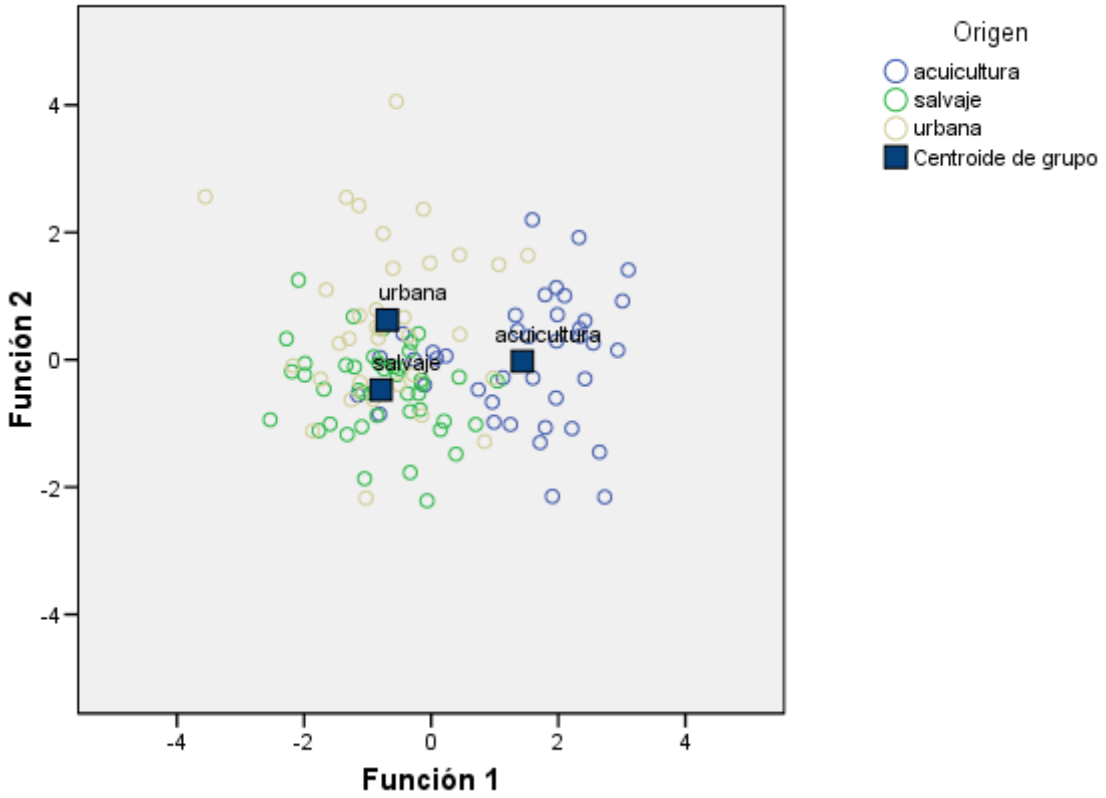


Figure 29. Landmark-based method: Discriminant Canonical Analysis of the overall head shape variation of Bogue along the first two canonical axes

The canonical discriminant function clearly separated the centroid of aquaculture group from the wild and the urban ones. The contrast of the function 2 to the function 1 was explained by lambda of wilks= 0.427

a) **b)** **c)**

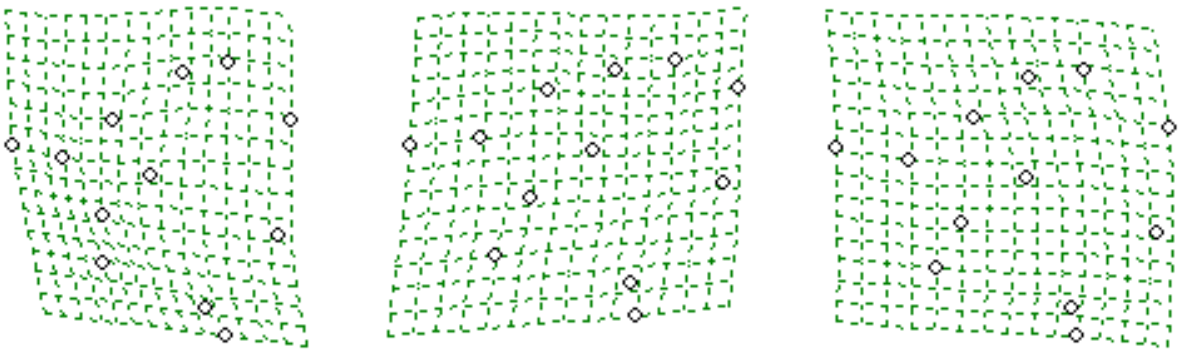


Figure 30. Grid deformation of Bogue's head from a) aquaculture zone, b) wild zone and c) urban zone

The grid deformation representing of Bogue's cephalic region from the aquaculture, wild and urban groups (Figure 30) confirmed that a great part of body differences among the 3 groups considered is indeed localized in the head region. In this sense, the aquaculture group stands out from wild and urban groups with a smaller head and differences in the jaw.

3.3 Surmullet body morphometry

In case of body morphometry of Surmullet, the program generated 34 relative warps. The Figure 31 represent the dispersion points of relative warps represented for the axes X=1 and Y=2.

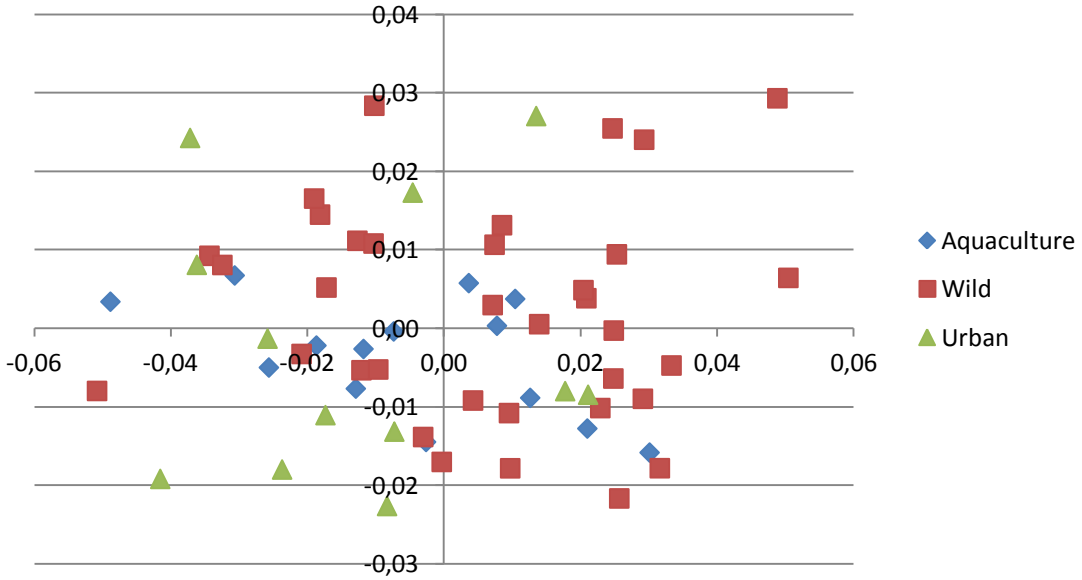


Figure 31. Relative warps ordination plot of Surmullet's Body, Axes (X=1; Y=2).

The cross validation test plotted on relative warps from the Surmullet's body performed a correct classification at 55.7%. In a more detailed analysis, the aquaculture group were correctly classified to 35.5%, and 42.9% of them were assimilated to urban group while 21.4 % were assimilated to wild group. Wild groups were correctly classified to the 54.3%, 22.9% of them were classified as part of urban groups while 22.9 % were assimilated to aquaculture group. Finally, urban group were correctly classified to the 75% and 16.7% of them were classified as wild group and 8.3% were classified as belonging to the aquaculture one.

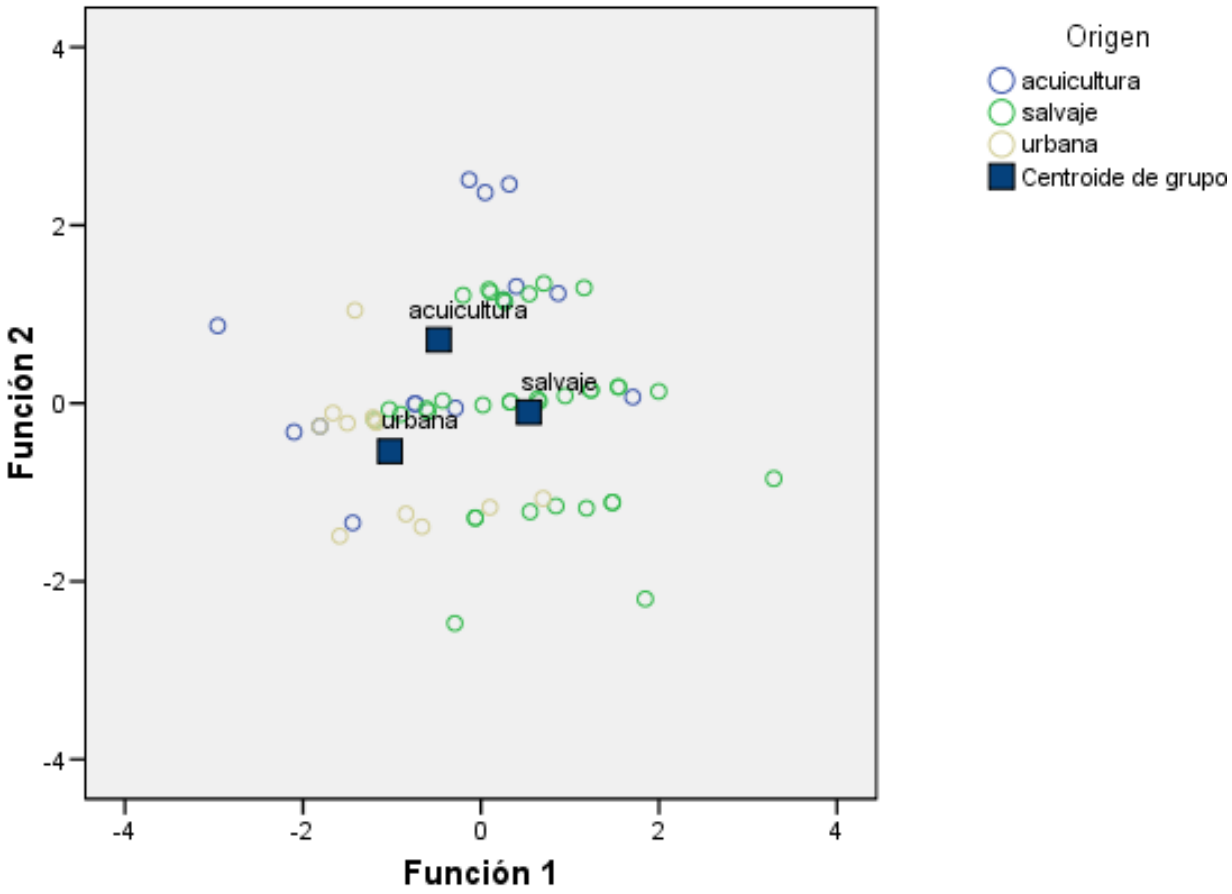


Figure 32. Landmark-based method: Discriminant Canonical Analysis of the body shape variation of Surmullet along the first two canonical axes

From the canonical discriminant function (Figure 32) there is separation of the centroid of aquaculture, wild and urban groups. The contrast of the function 2 to the function 1was explained by lambda of Wilks = 0.582

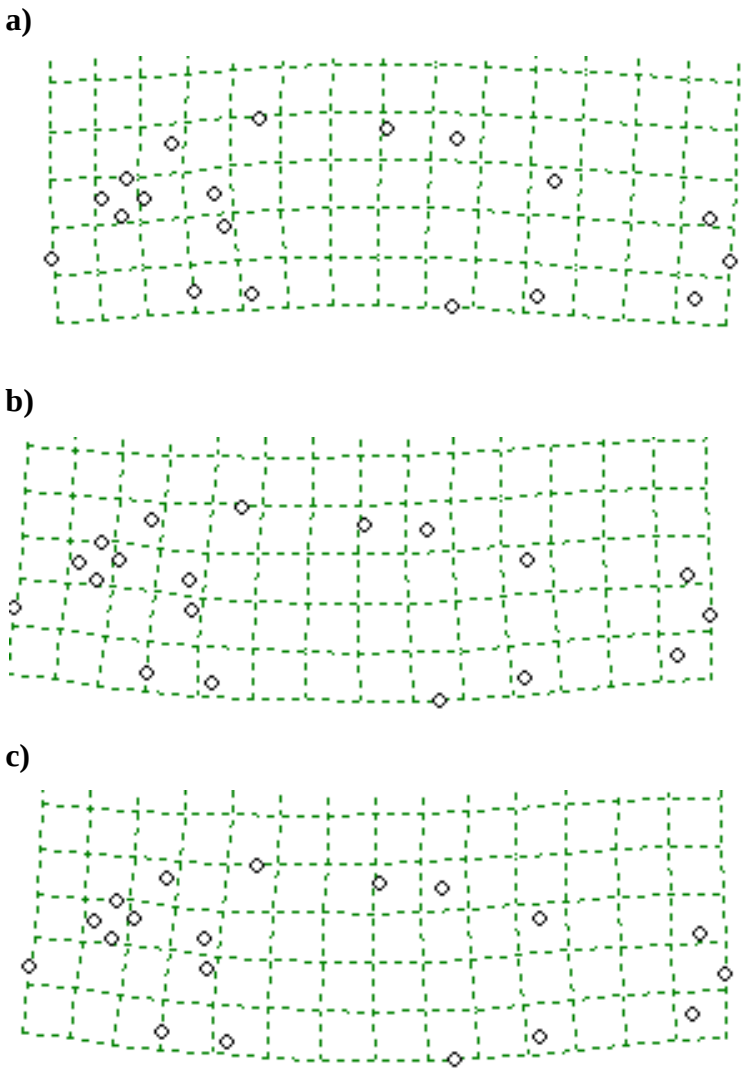


Figure 33. Deformation grid of body morphometry of the Surmullet from a) aquaculture b) wild and c) urban zone

The deformation grid obtained (Figure 33) of the shape morphometry for the 3 different groups of Surmullet did not obviously reveal a specific difference in the shape among the individuals studied. Nevertheless, in order to compare with previous results in Salema and Bogue, a morphological analysis on Surmullet's head was performed where 10 landmarks and semi-landmarks were added around the cephalic region (Figure 34).

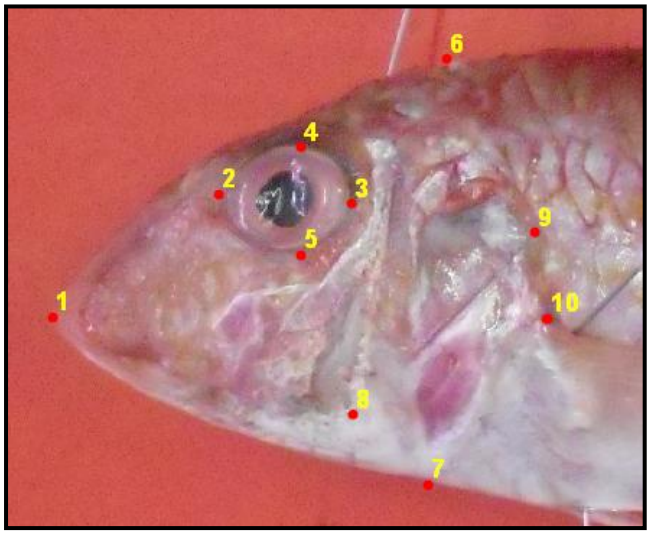


Figure 34. Landmarks addition in the cephalic region of Surmullet

The cross validation test plotted on relative warps from the Surmullet's head showed a correct classification estimated to 55.7%. The individuals belonging to the aquaculture group of Surmullet were correctly classified at 57.1%, while 14.3% of them were classified as wild and 28.6% were assimilated to the urban group. In the case of the individuals belonging to the wild group, 57.1% were correctly classified, 25.7% of them were classified as part of the urban group and 17.1% were assimilated to the aquaculture one. Finally, 33.3% of the fish belonging to the urban group were correctly classified, 33.3% were classified as aquaculture and 33.3% were classified as wild ones.

The morphometrical discrimination of warps of Surmullet's head is following the same tendency seen previously on the Surmullet's body. Also it is interesting to see that the urban species are equally similar to aquaculture and wild species, the wild species are more similar to urban species than to aquaculture species, and, somehow, the aquaculture species are more similar to urban species than to wild species.

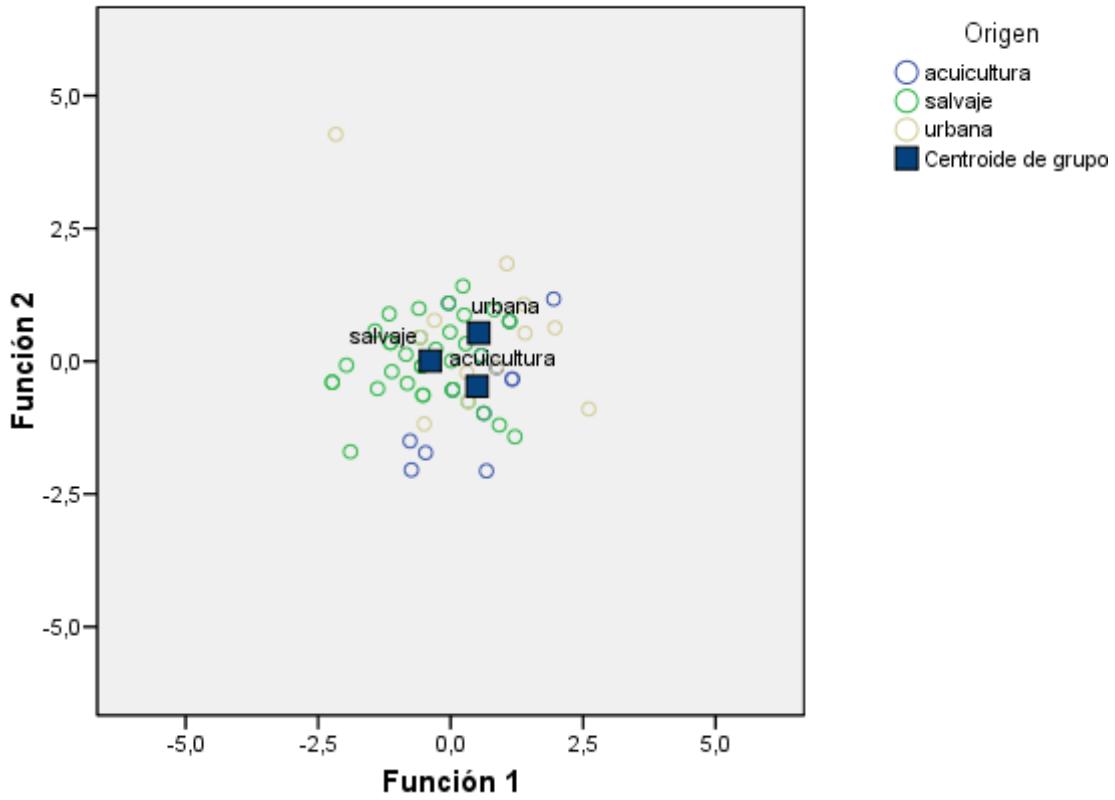


Figure 35. Landmark-based method : Analysis of the head shape variation of Surmullet along the first two canonical axes

The canonical discriminant function (Figure 35) scarcely separated the centroid of aquaculture groups from the wild and the urban ones. The contraste of the function 2 to the function 1 is explained by lambda of wilks=0.744.

a) **b)** **c)**

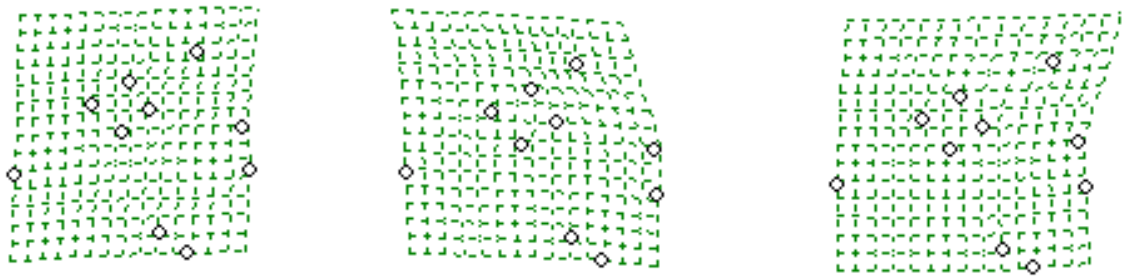


Figure 36. Deformation grid of the Surmullet's head from a) aquaculture b) wild and c) urban groups

The deformation grid showing the head shapes of Surmullet (Figure 36) didn't point out any visible difference in any part of the cephalic region as it was the case for the previous species.

3.4 Yellowmouth Barracuda's body morphometry

In the case of body morphometry of the Yellow mouth Barracuda, the program generated 20 relative warps. The Figure 37 represent the dispersion points of relative warps represented for the axes X=1 and Y=2.

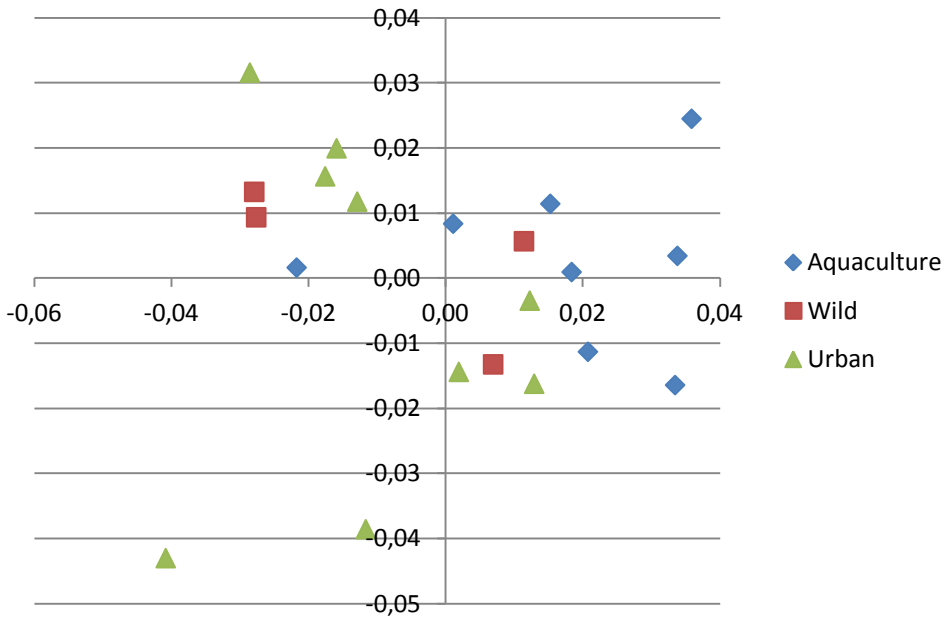


Figure 37. Relative warps ordination plot of Yellowmouth barracuda's body, Axes (X=1;Y=2)

The cross validation discrimination (Figure 38) plotted to warps of Yellowmouth baracuda's body were correctly classified to the 42.9%. Going into more details, the aquaculture group were correctly classified to the 75%, and equally classified as wild and urban groups to the 12.5%. Yellowmouth barracuda from urban group were correctly classified to the 42.9%, but another 42.9% of this group were assimilated as part of wild group, while 14.3% of this group were classified as part of aquaculture group. Finally, there is no sample of Yellowmouth barracuda from wild groups correctly classified in either group.

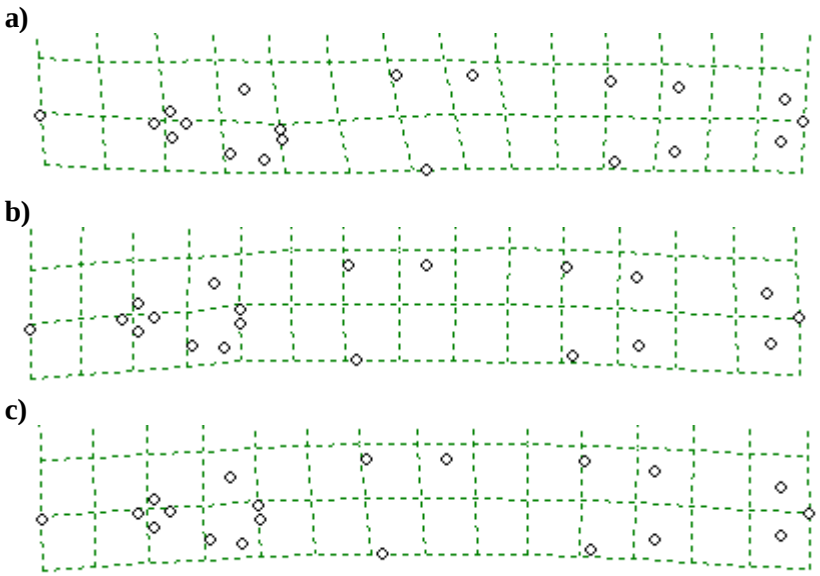


Figure 38. Deformation grid of the Yellowmouth baracuda's body from a) aquaculture b) wild and c) urban groups

The deformation grid of yellowmouth barracuda (Figure 38) did not indicate any difference in the morphometry of body among the aquaculture wild and urban groups.

4. Scales morphometry

4.1 Salema's scales morphometry

For the scale of the Salema, the program generated 10 relative warps. The Figure 39 represents the dispersion points of relative warps represented for the axes X=1 and Y=2.

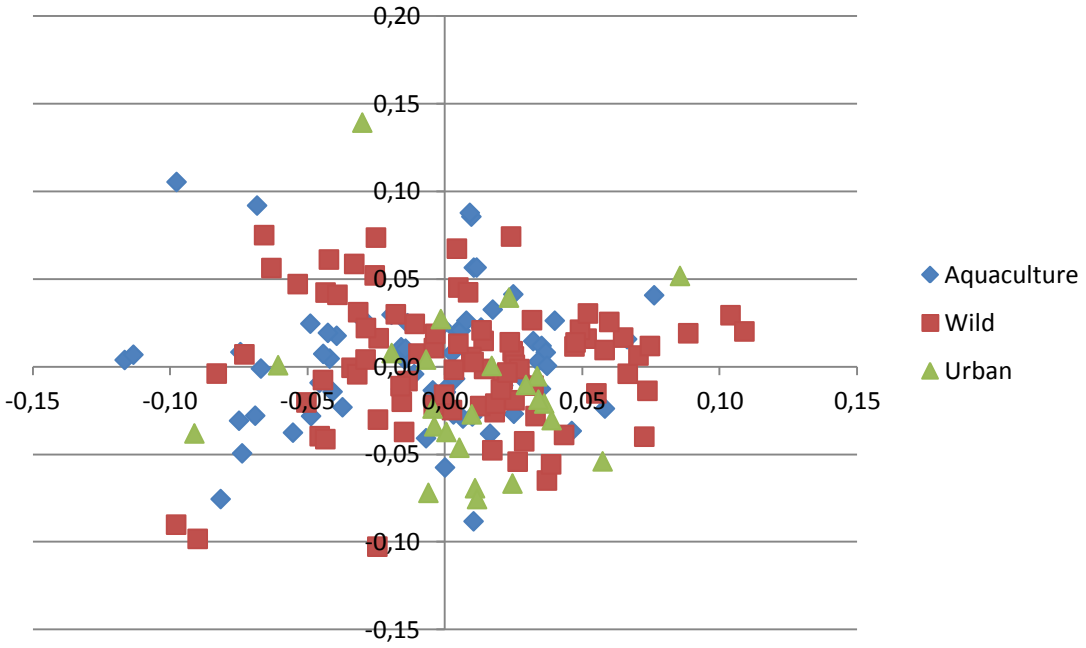


Figure 39. Relative warps ordination plot of Salema's scales. Relative warps (X=1;Y=2)

The cross validation discrimination plotted to warps of Salema's scales showed a correction to 54.2%. Thus, 47.8% of scales from the aquaculture group were correctly classified; 30% of them were classified as scales belonging to the wild group and 22% were classified as urban. In the case of the wild group, 52.3% of scales were correctly classified; 28% were classified as scales from the aquaculture group and 19.9% as scales from the urban group. Finally, 66.7% of scales from the urban group were correctly classified, 16.7% were classified as urban groups and 16.7% were classified as wild.

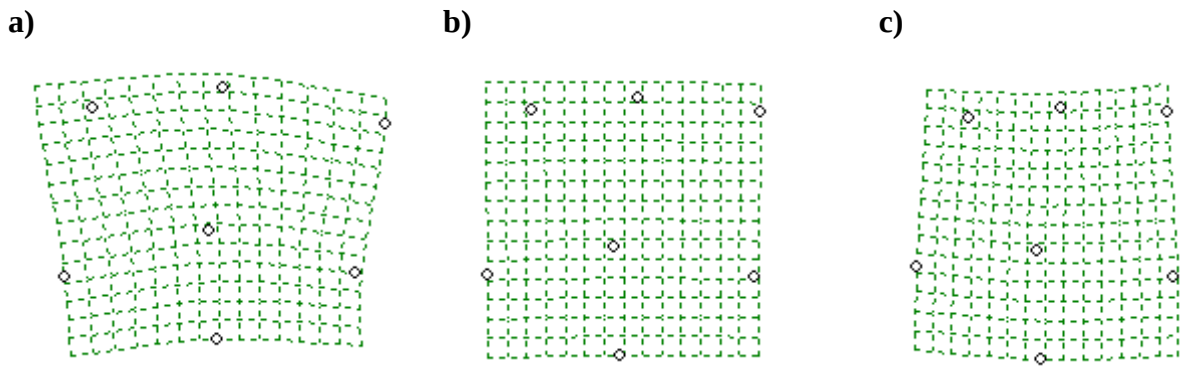


Figure 40. Deformation grid of Salema's scales from a) aquaculture b) wild c) and urban groups

The deformation grid of Salema's scales (Figure 40) showed a great diversity in the shapes of scales among the 3 studied groups of Salema. This variability is also observed within the groups, which do not allow to discriminate clear differences in relation to its provenance.

4.2 Bogue's scale morphometry

In the case of the analysis for the Bogue's scale, the program generated 10 relative warps. The Figure 41 represent the dispersion points of relative warps represented for the axes X=1 and Y=2.

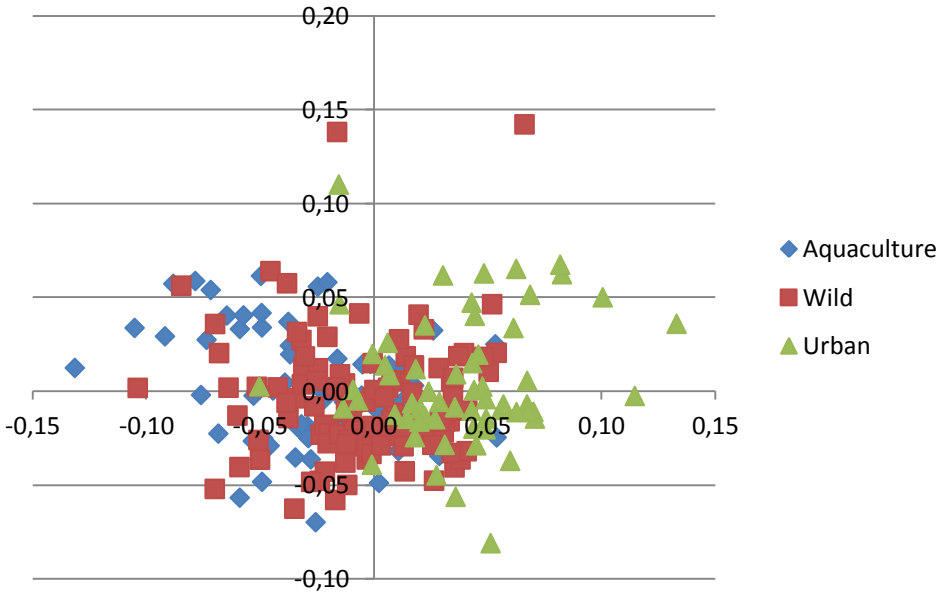


Figure 41. Relative warps ordination plot of Bogue's scales Relative warps (X=1;Y=2)

The cross validation discrimination plotted to warps of Bogue's scale had a correct classification estimated to 61.3%.

Bogue's scales from aquaculture groups were correctly classified at 58.3%, thus 25% of them were classified as scales of wild samples and 16.7% were classified as coming from urban samples. Scales from Bogue wild group were correctly classified at 54.6%, 29.6% of them were classified as scales from the aquaculture group and 15.7% of them as coming from the urban group. In the case of the scales from the urban group, up to 70% of scales were correctly classified, whereas 23.3% were classified as belonging to the wild group and 6.7 % were classified as coming from aquaculture.

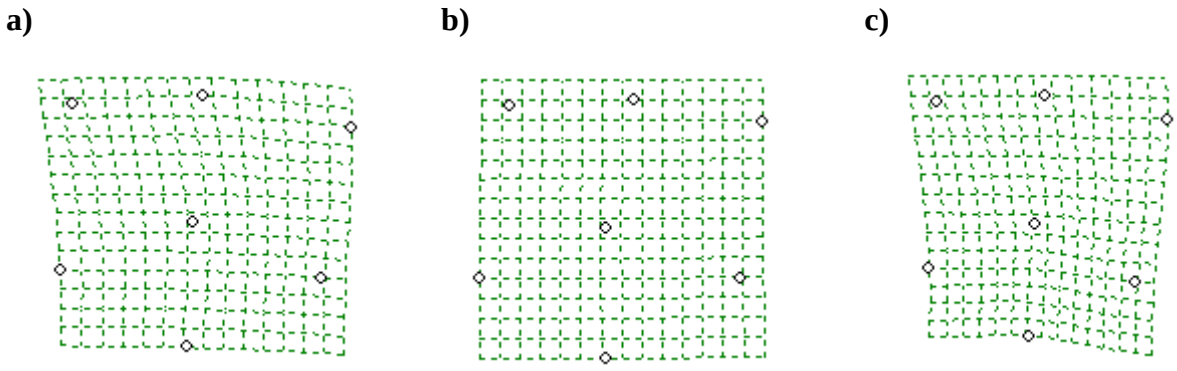


Figure 42. Deformation grid of Bogue's scales from a) aquaculture b) wild c) and urban groups

The deformation grid of Bogue's scale (Figure 42) pointed out an important difference between the scales of the different groups and also the observation of scales of each group made us deduce that there is also a difference of scale's shape within each groups.

4.3 Surmullet's scales morphometry

The program generated 10 relative warps. The Figure 43 represent the dispersion points of relative warps represented for the axes X=1 and Y=2.

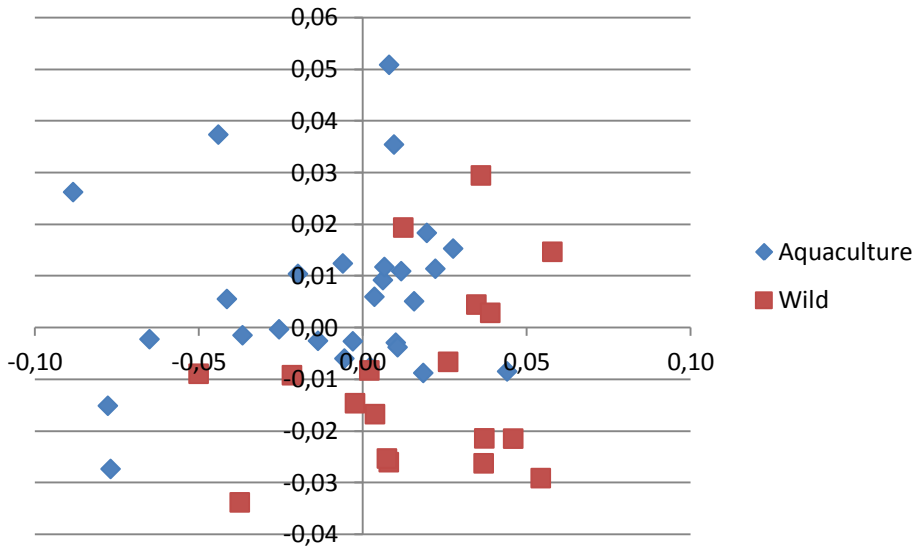


Figure 43. Relative warps ordination plot of Surmullet's scales. Axes (X=1 ;Y=2)

The cross validation discrimination plotted to warps of Surmullet's scales, the classification was correct at 89%. The comparison was made only between scales from aquaculture and wild groups of Surmullet, 89.9% of aquaculture scales were correctly classified and 10.7% were classified as wild ones. While 83.3% of scales from wild Surmullet were correctly classified and 16.7% of them were classified as aquaculture ones.

a) **b)**

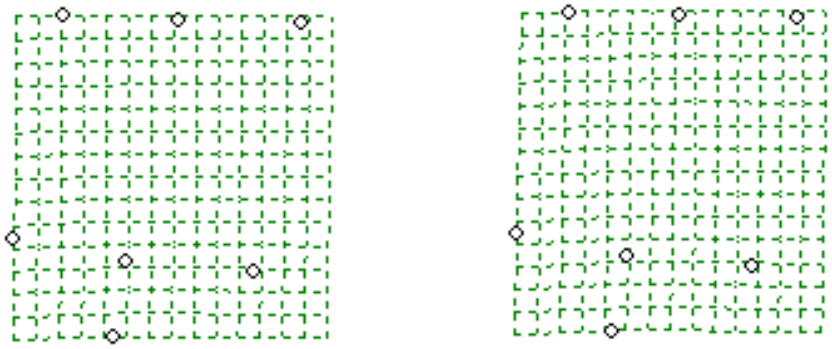


Figure 44. Deformation grid of Surmullet's scales from a) aquaculture and b) wild groups.

4.4 Yellowmouth barracuda's scales morphometry

The program generated 10 relative warps. The Figure 45 represent the dispersion points of relative warps represented for the axes X=1 and Y=2.

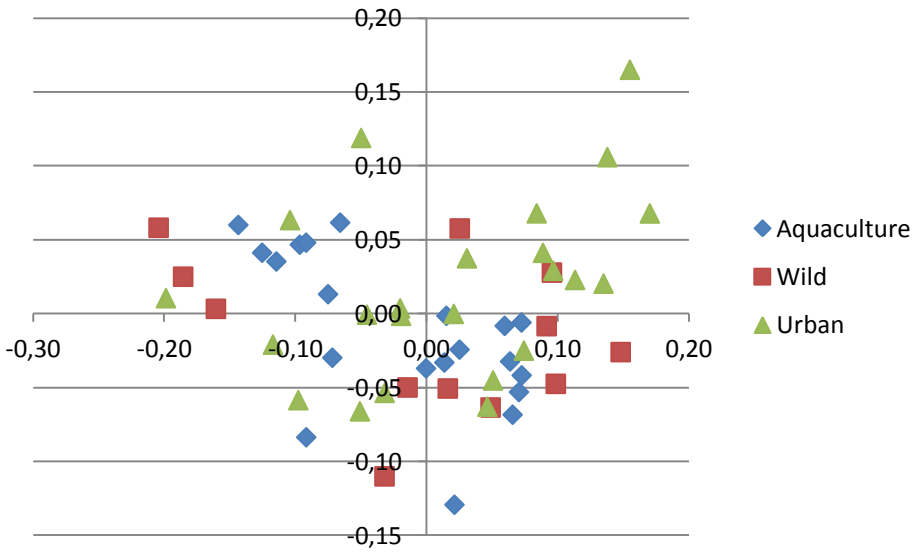


Figure 45. Relative warps ordination plot of Yellow mouth barracuda's scales. Axes (X=1;Y=2)

The cross validation discrimination plotted to warps of Yellowmouth barracuda's scales (Figure 46) was accurate on the 41.8%. Scales from aquaculture were correctly classified at 20%, whereas 50% of them were classified as scales from wild individuals and 30% of them were classified as scales coming from urban zone. Scales from wild groups were correctly classified at 58.3%, whereas 16.7% of them were classified as coming from aquaculture group and 25% of them were classified as coming from the urban group. Finally, 52.2% of urban scales were correctly classified, whereas 26.1% were classified as belonging to the aquaculture group and 21.7% were classified as coming from the wild group.

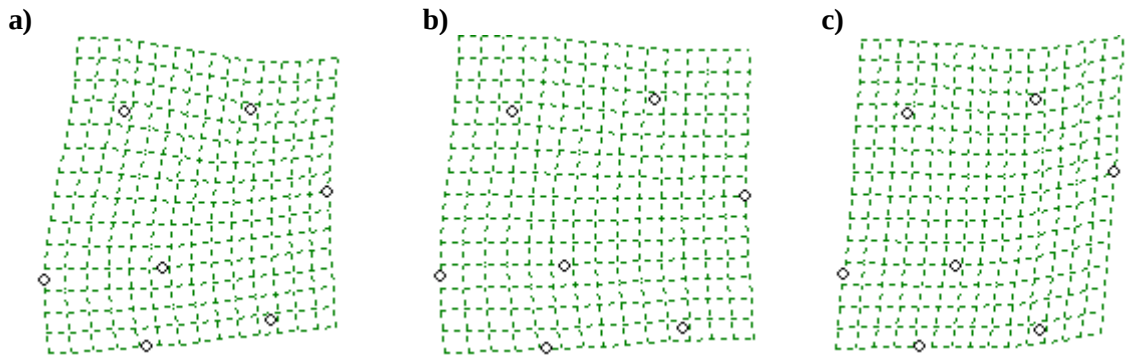


Figure 46. Deformation grid of Yellowmouth barracuda's scales from a) aquaculture b) wild and c) urban groups.

5. Lipid and Fatty acid analysis

5.1 ANOVA result's

The results involving: Total weight dry lipid, Total length, Total weigh, Eviscerated weight, Fulton's K, dry are summarized in table V.

ANOVA performed with multiple comparisons using the Tukey test. The homogeneity test showed that, the total length values were homogenous (P<0.05).

Table V. Total weight dry lipid, Total length, Total weigh, Eviscerated weight, Fulton's K of Salemas

	Aquaculture Group	*Wild Group*	*Urban Group*
Total dry weight lipid	8,34*± 1,65	5,51±0,60	6,66±2,48
Total length	31,33±8,76	25,43±1,31	25,37± 2,48
Total weigh	527,33± 374,84	248,72±24,54	268,8±137,53
Eviscerated weight	386,17±308,66	192,33±20,78	209,91±123,21
Fulton's K	1,40±0,09	1,51±0,13	1,55±0,13
Moisture	76,59±0,97	76,90±0,99	76,26±0,68

Table VI shows fatty acid composition (expressed as g/100 g fatty acid identified) and the significant differences among the three different groups (aquaculture, wild, urban) using a Tukey test (P<0.05).

Table .VI Fatty acids composition in the muscle of Salemas from aquaculture wild and urban groups

Fatty acids	*Aquaculture Group*	*Wild Group*	*Urban Group*
14:0	1,98±0,91	1,31±0,74	2,04±0,72
14:1n-7	0,043±0,027	0,036 ±0,034	0,035±0,019
14:1n-5	0,06±0,038	0,05±0,032	0,08±0,093
15:0	0,28±0,08	0,36±0,13	0,43±0,16
15:1n-5	0,33±0,78	0,042±0,037	0,26±0,50
16:OISO	0,028±0,01	0,025±0,01	0,024±0,008
16:0	19,40 ±6,27	19,22±2,72	22,59± 2,76
16:1n-7*	$2,023^{a}$ ±1,001	$0,98^{b}$±0,36	$2,05^{a}$±0,71
16:1n-5*	$0,08^{a}$±0,036	$0,048^{b}$±0,012	$0,055^{ab}$±0,015
16:2n-6	0,071±0,06	0,063±0,018	0,061±0,024
16:2n-4	0,40 ±0,06	0,58±0,05	0,51±0,024
17:0	0,13±0,05	0,14 ±0,07	0,21±0,11
16:3n-4	0,19±0,103	0,15±0,02	0,24±0,15
16:3n-3	0,099 ±0,13	0,021±0,012	0,038±0,050
16:3n-1*	$0,29^{a}$ ±0,24	$0,55^{b}$±0,096	$0,40^{ab}$±0,163

Fatty acids	*Aquaculture Group*	*Wild Group*	*Urban Group*
16:4n-3*	0,24[a]±0,208	0,50[b]±0,10	0,33[ab]±0,179
16:4n-1	0,08±0,047	0,13±0,06	0,09±0,046
18:0	8,20±1,67	9,11±1,06	7,47±1,37
18:1n-9*	15,72[a]±4,14	10,93[b]±1,75	13,81[ab]±4,41
18:1n-7	1,98±0,50	1,93±0,425	1,95±0,56
18:1n-5	0,149±0,083	0,134±0,059	0,10±0,035
18:2n-9	0,24±0,150	0,231±0,08	0,24±0,16
18:2n-6*	9,39[a]±6,23	3,067[b]±1,06	4,35[b]±1,96
18:2n-4	0,069±0,023	0,047±0,007	0,054±0,023
18:3n-6*	0,25[a]±0,13	0,25[a]±0,10	0,41[b]±0,11
18:3n-4	0,08±0,023	0,066±0,01	0,08±0,03
18:3n-3*	1,85[a]±0,40	0,86[b]±0,21	1,90[a]±1,03
18:3n-1	0,013±0,01	0,006±0,008	0,015±0,01
18:4n-3	0,98±0,58	0,52±0,41	1,54±1,47
18:4n-1	0,05[a]±0,02	0,09[b]±0,03	0,07[b]±0,03
20:0*	0,32[a]±0,078	0,246[b]±0,038	0,23[b]±0,073
20:1n-9*	0,73[a]±0,43	0,34[b]±0,04	0,39[b]±0,17
20:1n-7	0,16±0,06	0,11±0,02	0,14±0,02
20:1n-5	0,015±0,012	0,011±0,004	0,016±0,01
20:2n-9	0,12±0,09	0,09±0,042	0,13±0,15
20:2n-6*	0,66[a]±0,23	0,39[b]±0,13	0,39[b]±0,18
20:3n-6*	0,65[a]±0,33	1,30[b]±0,15	0,98[c]±0,2
20:4n-6*	10,73[a]±5,69	16,18[b]±2,45	13,72[ab]±3,001
20:3n-3	0,36±0,143	0,28±0,10	0,32±0,16
20:4n-3	0,811±0,32	0,86±0,33	0,88±0,50
20:5n-3	6,92±4,6	10,02±2,50	11,006±4,36
22:1n-11	0,23±0,10	0,19±0,09	0,17±0,028
22:1n-9	0,07±0,03	0,09±0,051	0,06±0,032
22:4n-6*	1,64[a]±0,94	2,85[b]±0,75	2,24[ab]±0,49
22:5n-6	1,03±0,53	1,58±1,25	0,41±0,22
22:5n-3*	3,93[a]±1,73	8,16[b]±1,63	5,20[a]±2,02
22:6n-3	6,79±4,89	5,73±4,26	2,16±0,83
n - 3	22±13,04	26,97± 9,58	23,81±10,80
n - 6	23,80 ± 13,84	25,70 ± 5,9	22,59±6,20
n – 9*	16,77±4,76[a]	11,70± 1,97[b]	14,63 ± 4,93[ab]
$n-3/n-6$	0,92 ± 0,94	1,05 ± 1,61	1,05 ± 1,74
EPA/ARA	0,64 ± 0,80	0,62 ± 1,02	0,80 ± 1,45
EPA/DHA*	1,02 ± 0,94[a]	1,81± 4,14[a]	5,08 ± 5,22[b]

Different letters within a row denotes significant differences (P<0.05) among sampling points.

The Salema from aquaculture area did not show a significant difference in the Fulton's K constant with respect to Salemas from wild and urban area. However, lipid content of Salema's muscle associated to the farm cage were significantly higher ($P<0.05$) when compared to Salemas from either wild or urban areas.

The ANOVA performed on fatty acids content in Salemas from the different groups showed significant differences in fifteen fatty acids.

- ✓ Palmitoleic Acid (16:1n-7) was significantly higher ($P<0.05$) in muscle of Salemas from aquaculture and urban groups than salemas from wild groups.
- ✓ Oleic acid (18:1n-9) was significantly higher ($P<0.05$) in muscle Salemas from aquaculture groups than Salemas from wild groups, although there was no difference with Salemas from urban groups.
- ✓ Linoleic acid (18:2n-6) were significantly higher ($P<0.05$) in Salemas from aquaculture groups than Salemas from wild and urban groups.
- ✓ Gamma linolenic acid (18:3n-6) were significantly higher ($P<0.05$) in Salemas from urban groups than Salemas from wild and aquaculture groups.
- ✓ Alpha linolenic acid (18:3n-3) were significantly higher ($P<0.05$) in Salemas from aquaculture groups than Salemas from wild groups, the highest value was found in Salemas from urban groups which showed a significant difference ($P<0.05$) in comparison with the wild ones.
- ✓ Arachidic acid (20:0) were significantly higher ($P<0.05$) in Salemas from aquaculture groups than Salemas from wild and urban groups.
- ✓ Monoenoic acid (20:1n-9) were significantly higher ($P<0.05$) in Salemas from aquaculture groups in Salemas from wild and urban groups.
- ✓ Eicosadienoic acid (20:2n-6) were significantly higher ($P<0.05$)in Salemas from aquaculture groups when compared to Salemas from wild and urban groups.
- ✓ Dihomogamrnalinolenic acid DGLA (20:3n-6) were significantly ($P<0.05$) higher in wild groups of Salemas than from aquaculture and urban ones, moreover, the urban groups showed also a significantly ($P<0.05$) higher value of this fatty acid than in aquaculture ones.
- ✓ Ecosapentaenoic Acid EPA (20:5n-3) did not show significant difference among the three groups of Salema.
- ✓ Arachidonic acid (20:4n-6) were significantly ($P<0.05$) higher in wild groups of Salemas than those observed from aquaculture groups.

- ✓ Docosapentaenoic acid (22:5n-3) were significantly (P<0.05) higher in wild groups of Salemas than in aquaculture and urban ones.
- ✓ Docosahexaenoic acid DHA(22:6n-3) did not show any significant variation among the three groups of Salema.

5.2 Principal component analysis (PCA)

The PCA analysis of fatty acid content of Salemas muscles and the total body weight (Figure 47) did not clearly separate the groups from the different origin and did not show any cloud distribution tendency for each group of Salemas.

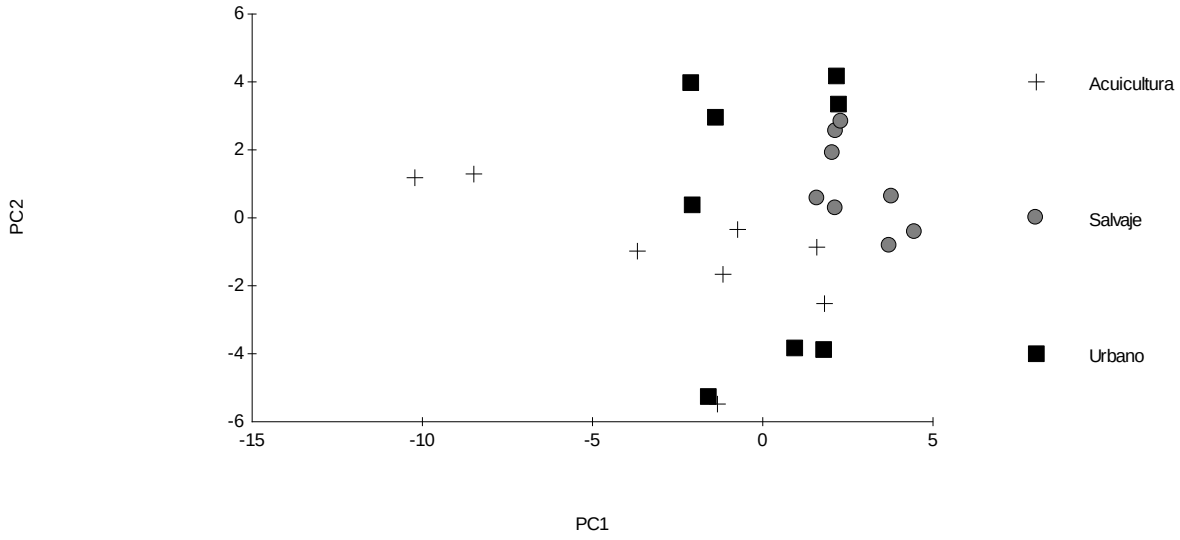


Figure 47. The PCA analysis of fatty acid content of Salemas muscles and the total body weight

5.3 Simper's results

The dissimilarity between aquaculture and wild groups were estimated to 24.03%, while it was calculated for aquaculture and urban groups as equal to 23.15%. Finally between wild and urban groups the dissimilarity was estimated to 18.40%.

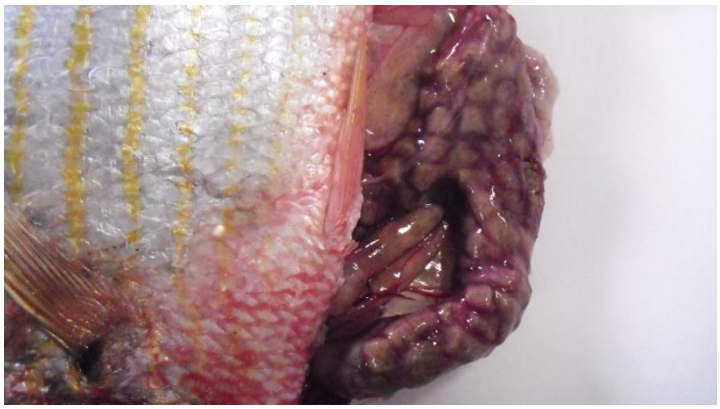


Figure 48. Feed pellet encountered during the dissection process in the stomach of Salema *Salpa salpa* from aquaculture groups

Discussion

1. Otolith weight

Regarding the overall results related to the weight of the analized otholits, there is a tendency of heavier otoliths in the wild groups of Salema, Yellowmouth Barracuda and Surmullets compared with the otoliths of the same species coming from the aquaculture groups; these results are similar to the ones obtained by Secor & Dean (1986) and Pawson (1990) in *Sardinella aurita*. In this sense, our results confirmed by previous studies suggest that slower-growing individuals may have larger otoliths than faster-growing individuals of fish with similar sizes or ages. Huuskonen & Karjalainen (1998) hypothesized that there were at least two component governing fish otolith growth, one independent and one dependent on somatic growth, the first is related to maintenance metabolism and the second to feeding-induced growth. Larger otolith in slower-growing fish have a higher ratio of mineral to protein in their otoliths, that's why they produce heavier and also thicker otoliths (Templeman & Squires, 1956; Radtke et al., 1985). This change in the deposition of otolith material supports the Daily Increment Packing (DIP) hypothesis proposed by Secor & Dean (1989), which states that as long as suboptimal conditions are not prolonged, otolith growth will continue independently of somatic growth. The breakdown of this relationship continues until some point in time where otolith deposition ceases due to the lack of energy reserves. The amount of time it takes for these changes to manifest are variable and dependent on the species and energy reserves of each individual fish (Hoff & Fuiman, 1993).

This change in otolith weight observed between the different groups of the same species and also observed in most of the species studied, can be attributed to a change in the nutritional regime of the fish, in fact, Hussy & Mosegaard (2004) made a conceptual model for otolith where in periods of reduced fish feeding they predicted a more translucent otolith growth, which was later confirmed by Hoie and co-workers (2008) working with juvenile cod *Gadus morhua*. It was observed that alternating periods of severe food reductions with other intense feeding changes influenced the otolith opacity which means that feeding can be a responsible factor of growth structure changes in the otolith features.

Therefore, it seems that otolith weight is closely related to somatic growth process (Zorica et al., 2010), which explains why otolith weight is most sensitive to variations in growth

rate and most closely related to changes in fish metabolism (Secor & Dean, 1989; Pawson, 1990; Begg et al., 2000).

Besides in the work of Zhag and co-workers (1995), otoliths of the Chinook salmon from the Cowichan hatchery were smaller than those from the wild species since for a given amount of energy by exogenous feeding, it was observed that the Cowichan hatchery-reared Chinook convert less energy for otolith growth, resulting in relatively small otoliths. That's why the otolith weight was considered as indicator of relative growth difference (Strelshek et al., 2003)

In our case it was observed that otoliths from urban groups of Salemas and Bogues were heavier than the ones from the aquaculture groups of the same species. At the same time, the otolith weight from Salemas from wild and urban groups were very similar, although it was not the case with the bogue's otoliths. This could be interpreted in different ways, since that fishes from San Cristobal (at the entrance of Las Palmas de G.C. city) were collected next to urban emissaries where domestic and organic substances are rejected to open sea (Grafcan, 2012) [3]

The constancy of otolith weight between urban and wild Salemas could demonstrate that despite the pollution observed in the area of sampling, it did not influence the nutritional regime of the fish (herbivorous fish species). In fact, this organic pollution generated an eutrophization of the waters via more nutrients availability and, thus, of phytoplankton abundance, which possibly did not affect the feeding regimes of the associated Salemas. While the differential in otolith weight of Salema from the aquaculture group compared to wild and urban groups could be due to the large availability of macroalgae in the cages structures (ropes, nets and other submerged structures) as consequence of nutrient leaching from the aquafeeds used, and also, from the direct feeding of Salemas on the rest of the pellet wasted from the aquaculture cages.

It seems from our results that the use of otolith weigh as indicator for fish species associated to aquaculture cages would be of a great interest for investigation related to the impact of aquaculture cages on the wild fish fauna, apart from being easily applicable and cheep. Nevertheless, it is necessary to have a larger sampling strategy to afford more significant data from the otolith weight but also about the urban pollution existing in the sample zones for a better interpretation of the results.

2. Otolith morphometry

The shape of the otolith were considered by many authors to be an ideal marker for fish population affiliation and has been used to differentiate between stocks as a natural marker in stock identification studies (Campana & Casselman 1993; Begg & Brown 2000), but it is important to consider the factors that may affect the shape variability (Canas et al., 2012).

Our results of the analysis of otolith shape of Salemas and Bogue, belonging to aquaculture urban and wild groups, did not show any difference whether in the wavelts of the otolith or by the statistical analysis. So we can conclude that there is no otolith shape variation between the three groups (aquaculture, wild and urban) of both Salema and Bogue.

Even if the factors influencing otolith shape have not been fully understood (Burke et al., 2008), there is many studies that consider the otolith shape, among other morphometric traits, as a characteristic that reflects a combined effect of genetic variation and local environmental factors such as depth and water temperature (Lombarte & Lleonart, 1993; Swain et al., 2005; Schulz-Mirbach et al., 2008). In *S. aurata,* important differences in otoliths shape were found within geographical origins, which could be explained by either genetic or morphological differences of Mediterranean Sea stocks (Arechavala–Lopez et al., 2012).

Since the otolith shape is commended by environmental and also genetic factors. We will discuss both of these hypotheses: It had been reported that environmental factors could influence the morphometry and specifically the otolith shape in case of environmental segregation between compared stocks, the stock separation counting for a life history. For instance in De Vries et al. (2002) it was possible, to distinguish individuals from eastern Gulf and Atlantic stocks of king mackerel using otolith shape data and to estimate stock composition in the mixed-stock fishery. Also, the analysis on internal otolith morphometric differences between the eastern and western Georges Bank haddock spawning components reflected that the differences didn’t have genetic basis but reflected phenotypic characteristics indicative of stock separation during life history (Begg et al., 2000; Begg et al., 2001), which mean that the variation in the shape of otoliths assists in distinguishing between groups of fish that are at least partly separated and inhabit different environments thereby remaining unaffected by short-term changes in fish condition(Bird et al., 1986; Campana & Casselman, 1993; Begg and Brown, 2000; Begg et al., 2001)

In the study of Hutchinson et al (2001) there were differences the otolith shape between the spawning groups of cod from Moray Firth and Viking, which appear to be reproductively isolated and show a separation during life history. In our study, the aggregation of fishes around the aquaculture cages and emissaries is limited in time, temporally stable for weeks to months (Dempster et al., 2004). Then, we can't assume that there is enough environmental factors separating the groups of Salema and Bogue associated to aquaculture cages or to emissaries that could influence the otolith shape which clarify the reasons why we didn't find differences in the otolith shape of our samples.

On the other hand genetic differences between populations can also explain changes in the otolith shapes (Jonsson &L'Abe´e-Lund 1993; Simoneau et al., 2000).Differences in otolith shape among spawning groups may have a genetic component. For some species, the level of classification success obtained using otolith shape analysis increases with the extent of genetic discreteness or geographic separation displayed by study groups (Castonguay et al., 1991; Friedland & Reddin, 1994), implying that otolith shape variation is determined by genetics.

In the study of Cardinal et al. (2004) genetically driven differences in otolith shape of cod from separate stocks were detected as signal of genetic differences between populations that can influence the shape of otoliths in the absence of any other growth related differences (Galley et al., 2006) this occur because the production of carbonic anhydride and otolin in otolith might be affected by genetic processes that contribute to the regulation of the otolith mineralization (Merigot et al., 2007)

According to Lombarte & Lleonard (1993) genetic conditions regulate the form of the otolith. Analogically, in the Canary Islands, there is no study on Salema (Mendez – Villamil et al., 2001) or Bogue populations that reported genetic differences within each species. Then we can say that our results are going in the same direction with this hypothesis since we did not detect differences in otolith shape in Salemas and Bogues belonging to aquaculture, wild and urban groups. Therefore we can suggest that we are in the presence of the same population of Salemas and Bogue which have the same genotypic characters being associated to aquaculture cages, emissaries or from the wild.

Our results suggest that the use of otolith shape analysis is not powerful to detect fish population associated to aquaculture cages: firstly because this character could be

controlled by both environmental and genetic factors which make difficult to identify the real origin of shape otolith variation.

On the other hand, this parameter needs a life history segregation between fish population of the same specie to produce effect on the otolith shape, this effect could be genetically commended and controlled by environmental parameters acting during long-term periods.

3. Body Morphometry

3.1 Discrimination analysis

The results of classification of warps from Salemas's body show that all aquaculture, wild and urban the groups were highly well classified, whereas Salemas from aquaculture groups the best classified although 11.1% of which were similar to wild ones. In addition, Salemas from urban groups were more similar to aquaculture groups than to wild groups.

Equally, the classification of the Bogue body's warps showed that the highest classification rate were seen in the aquaculture group, also the individuals belonging to the urban group were more similar to the wild group than to the aquaculture ones.

In the case of the Mullet classification of warps based on discrimination analysis was lower than in Bogue and Salemas, with individuals of the urban groups best classified, while 23% of the individuals belonging to the wild group were classified as urban groups, and the aquaculture group classification were the weakest with only 35.5%.

Concerning the Yellowmouth barracuda even if the best classified individuals were those belonging to the aquaculture group that seems to be different from the rest of the groups. It was observed that the urban and the wild groups had the same classification.

The tendency, seen in the 4 species studied by cross validated classification, is that the aquaculture individuals are the best classified group and show a low assimilation to the urban and wild groups. Meanwhile, the urban and the wild groups seems to have a closer similarity, for instance, the Yellowmouth barracuda showed the same classification for both urban and wild groups.

3.2 Deformation Grid

The deformation grid of the Salema and Bogue showed geomorphometrical differences for individuals belonging to the aquaculture groups comparing to wild fish groups.

Meanwhile, the urban and wild groups did not practically show any geomorphometrical differences.

The observed morphological differences in these 2 species were related to the anterior part of the body specially the head, where the cephalic region from aquaculture groups of Salemas and Bogues were reduced compared to wild and urban groups. These differences were seen mainly in the proportion of the eye and the distance between the base of the month and the opercula while the body and the peduncle zone did not show any difference.

The deformation grid of Surmullet and Yellowmouth barracuda did not show any morphometrical differences on the body.

In the literature no geomorphometrical studies were performed on fishes associated to aquaculture cages. Our research is the first contribution to this morphological topic. Therefore, taking into account the environmental similarity between conditions of fishes associated to aquaculture cages and fishes reared in cages, we compared our results to studies achieved to discriminate between wild and reared fishes using body shape differentiation, where differences in behavior, morphology and physiology between the hatchery and wild fishes were studied (Gross, 1998; Rogdakis et al.,2003)

Our results agrees with those of Vehanen & Huusko (2011) who studied the morphological differences in the rearing environment of juvenile *S. trutta* between wild and reared species of similar age and of same gene pool. These differences were mostly related to the anterior part of the body, where head shape among the wild fish was elongate compared to the hatchery fish. Also, in the study of Arechavala-Lopez and co-workers (2011), who found that most of the differences between reared and wild Gilthead Seabream and European Sea Bass are located primarily in the head and anterior region of the fish body. In addition, Busack and co-workers (2007) compared wild adult with first generation hatchery of Chinook Salmon and found that hatchery fish have shallower bodies than wild fish, being the main difference localized in the head and the anterior body regions.

Morphological changes observed in aquaculture groups are obviously due to diet change among fishes from aquaculture groups compared to fishes from wild groups. Since we suspect that fishes associated to aquaculture cages are directly or indirectly feeding from the lost pellets wasted from the aquaculture cages. As a matter of fact, some individuals of Salema collected near the aquaculture cages were observed, during the process of dissection with pellet in its stomach (Figure 48), we suspect that this could lead to a radical transformation of the nutritional regime of this species. The influence of feeding behavior

on morphology have been widely investigated (Cavalcanti et al., 1999; Kassam et al., 2004) Currens and co-workers (1989) found that the feeding regime in the hatchery affected the morphometric measures of Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) and rainbow trout *Oncorhynchus mykiss* (Walbaum 1792).

Moreover the morphometric difference observed in the cephalic region of Salemas and Bogas from aquaculture groups could probably be explained by the change in trophic regime. Salema is an herbivorous species feeding on benthic macroalgae and an essential link to higher levels of the food web as consumers of primary production (Jadot et al., 2006) and the Bogue is an omnivorous species with preference of phytoplankton material but also feeding on diverse invertebrates preys (Sergious &Karpouzi., 2002). Thus, several studies have shown that changes in the feeding habits could affect the shape of the head (Myer, 1987; Hard et al., 2000; Hegrenes, 2001). In the perch, *Perca fluviatilis* L. 1758, selection of different prey items causes diverging head morphologies (Heermann et al., 2007). Wintzer & Motta (2005) reported a longer, more fusiform head shape among wild largemouth bass *Micropterus floridanus* (LeSueur 1822) compared to hatchery-reared individuals, and they determined that this was due to the feeding behavior.

In Kassam and co-workers (2002) substantial morphometric differences between two sympatric species *Ctenopharynx pictus* and *Otopharynx sp*. were localized in the head region: the mouth of *C. pictus* is larger than that of *Otopharynx sp*., which allow the two species to exploit different trophic levels. Later, Kassam and co-workers (2004) distinguished between zooplankton and algal feeders of cichlid fishes by quantifying the shape of several bony elements from head region and were able to distinguish among those groups, by revealing trophic morphological variation between species within each trophic guild.

Meanwhile, the urban groups of Salemas and Bogues did not show any obvious shape difference compared to wild species. Since we suspected earlier that the feeding regime transformation in aquaculture groups were mostly responsible of morphological body changes, this leads to deduce that samples collected from urban waste water areas did not change their feeding behavior. In general, the introduction of anthropogenic nutrients into coastal waters may produce an increase in the concentration of nutrients, which leads to increase in the phytoplankton production and biomass (Pullin et al., 1993). With this nutrient enrichment, the availability of macronutrients (nitrogen, phosphorus) is higher and

primary production by algae and macrophytes is stimulated (Meyer-Reil & Koster, 2000). We suppose that this enrichment only provided a surplus of nutrient but did not influence the feeding regime of the Salema and, in some way, also for the Bogue belonging to the urban groups. This hypothesis could explain why the morphometrical shape of these two species changed in aquaculture groups and showed spares difference in urban groups with respect to the wild group of species.

4. Scales morphometry

The morphological characteristics of fish scales proved in many studies to be a useful tool to discriminate species of the same genus as well as populations (Poulet *et al*., 2005; Jawad et al., 2005; Jawad & Al Jufaili, 2007; Taylor & Piola, 2008). More specifically, the scale morphometry represent an important phenotypic characteristic for fishes as they interact with the surrounding environment through scales by its potential influence on swimming performance (Long et al., 1996; Graduno-Paz et al., 2010; Ibanez et al., 2012).

From our results observed in Bogue, Salema, Surmullet and Yellowmouth barracuda belonging to the aquaculture, wild and urban groups, we noticed that there was too much shape variability among the scales sampled. This was observed by the deformation grid of the scales of species belonging to all the studied groups. In fact it was not possible to represent a typical scale shape for each group of specie. Besides, the cross validation discrimination was not able to show any classification tendencies. Therefore, it did not allow us to discriminate scales with respect to their group's origin; although, this large variability of the scale morphology could be considered as an artifact since the scales were removed from the posterior part of the body of all the species. Observing the results obtained of Ibañez and co-workers (2009) in his study to discriminate the scales of 3 teleostean fishes *M. cephalus*, *M. curema* and *Dicentrarchus labrax*, the scales used to shape analysis were removed from 9 body areas located in the Anterior, Central and Posterior zones of the bodies. They showed that the posterior areas had the greater shape variability and appear to be less effective in discrimination. The authors preferred the scales from anterior and central zones, this was also confirmed by Matondo and co-workers (2010) where by analyzing shape scales between male and female of *Upeneus vittatus* he showed that the most variability were found from scales in the posterior area above the dorso-lateral line. Clifford and co-workers (2011) found also much variability analyzing the intraspecific scale variation in selected regions of the Yellowtail Parrotfish

Scarus hypselopterus specially the central and the posterior part as shapes varied from circular to irregular and the sizes varied from small to large.

The shape variations along the longitudinal and transversal axes of the body are related to the curvature of the fish in the posterior region, since body surface area decreases substantially and curvature area increases. The scale rows fit into a smaller surface area by reducing the size of the scales and changing their shape. They become compressed along the dorso-ventral axis thus allowing more space for adjacent rows of scales (Ibañez et al., 2009)

The intraspecific variability encountered in scales shapes could not allow us to use the shape scales analysis as a tool for discrimination of the different groups of fish species, then we could not assume if this bio-indicator is useful or not, since it is very much related to the area where the scales should be collected. More samples of scales from other areas of the body of fishes could help us identify the utility of analysis of scale's shape in determining the origin of individual samples.

5. Lipid and Fatty acid analysis

There were some influences of the site of sample collection on the fatty acid profile of the muscle of Salema fish. The fatty acid analysis on Salema belonging to wild urban and aquaculture groups showed a differential profile, those animals being different from those taken in non-human influence sites. The proportion of palmitoleic acid (16:1n-7), oleic acid (18:1n-9), linoleic acid (18:2n-6) and alpha linolenic acid (18:3n-3) were significantly higher in aquaculture samples with respect to the samples of wild Salemas. Although, the Arachidonic acid (20:4n-6) and the Docosapentaenoic acid (22:5n-3) content of Salema muscle were significantly higher in wild Salemas with respect to aquaculture species. The EPA (20:5n-3) did not show significant difference among the three groups of Salemas but still it was observed that this FA presented lower proportion in aquaculture groups then in wild ones. Also the DHA (22:6n-3) did not show significant difference and seem to be stabilized among the three groups of Salemas.

When observing the urban group, the Gamma linolenic acid (18:3n-6); Palmitoleic Acid (16:1n-7); Alpha linolenic acid (18:3n-3) presented higher proportion than those from wild and aquaculture groups.

The PCA did not bring any additional information about the fatty acid profile pattern of Salemas belonging to the three groups. Although the Simper analysis showed that

dissimilarity in fatty acid profile in aquaculture groups is equally proportionate while compared to urban and to wild groups and also higher than the dissimilarity observed between wild and urban groups. This indicate that the aquaculture groups of Salemas present globally different fatty acid profile, while the urban and the wild groups show less difference in their fatty acid profil.

Several studies have highlighted that the FA composition of wild fish populations (Skog et al. 2003, Fernandez-Jover et al. 2007, 2010) and of other associated fauna to aquaculture cages, such shrimps (Olsen et al., 2009), can be altered as a consequence of food pellets that are not consumed by the cultured fish and are lost from the cages (Fernandez-Jover et al., 2011b). What is obvious from our results is that the FA profile of the aquaculture Salema population presented an increase in FA such as which are 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-3 with respect to those from the wild population. Some of those fatty acids such as Linoleic acid or alpha linolenic acid have been named as "terrestrial fatty acids", since their presence in marine organisms is lower than in terrestrial organism, and are characteristic of different vegetable oils used in fish feeds, such as soybean oil or sunflower oil, both rich on linoleic acid, or linseed oil and rapeseed oil with high amount of alpha-linolenic acid (Sales & Glencross, 2010).

The Salema collected around the sea cages presented a feeding behavior similar to the one mentioned for other fish species associated to aquaculture cages, as it has been demonstrated that cage aggregating species have changed their diet while resident around farms. This was explained by their active consume the lost particulate organic matter in the form of uneaten food pellets and faeces that fall from the cages (Fernandez-Jover et al. 2007, 2008; Dempster et al., 2009) The increasing utilization of vegetable oils in the production of aquafeeds results in an increase of those so-called "terrestrial fatty acids" in cultivated fish, that shows higher levels of mainly linoleic acid (Fernandez-Jover et al., 2011a). For instance, the use of corn or soya oils in pellet production gives a high proportion of oleic acid (18:1n-9), linoleic acid (18:2n-6) and linolenic acid (18:3n-3) in commercial diets and therefore influence the FA's profile of reared species consuming the commercial feed thus it was reported that Salmon and cod fed on diets based on plant oils have been shown to have elevated levels of18:2n-6 and18:1n-9 in their tissue (Bell et al., 2000 & 2001).

Our results agrees with those of Skog et al. (2003) that studied the feeding behavior of wild saithe *Pollachius virens* around salmon farms in a Norwegian fjord. It resulted that

FA's profiles of this specie were similar to the food pellets used at the farm and showed an increasing levels of linoleic and α-linolenic acids.

But, is this change of fatty acids usable as bio-indicator of an aquaculture imprint? Previous studies have discussed the use of FA as dietary markers (Iverson et al., 2004) or markers of aquaculture influence due to the change of the FA composition of associated fauna like sea-urchins (Cook et al., 2000), mussels (Gao et al., 2006), shrimps (Olsen et al. 2009), fish (Skog et al., 2003, Fernandez-Jover et al., 2007) and also in sediment (Samuelsen et al., 1988; Henderson et al., 1997).

Dalsgaard et al. (2003) define trophic markers as a compound whose origin can be easily and unequivocally identified, it is inert and does not harm the organisms, is metabolically stable and not selectively processed, and transfers from one trophic level to the next in both a quantitative and qualitative manner. In this sense, Olsen et al. (2009) considered that only linoleic and α-linolenic acids can be used as clear aquafeed markers in the northern shrimp *Pandalus borealis.* The advantage of the use of FA's as bio-indicators is due to the rapid change in the FA profile of fish associated to sea cages, with a strong increment of linoleic acid and diminished levels of EPA and in the n-3/n-6 PUFA ratio (Fernandez-Jover et al., 2011b). Also, because it has been estimated that 3 to 4 month are sufficient time to provoke substantial changes in fatty acid composition of the tissues (Fernandez-Jover et al., 2007) which is an important criterion as fish associated to aquaculture cages are known to have a limited temporally residence around aquaculture cages, but still their FA's profile would be affected. Although it is recommended to concentrate on individual FA's, so called 'Key FAs', which may act as discriminators between different fish and avoid 'noise FAs' that do not aid in discrimination (Fernandez-Jover et al., 2011a) in our study the Linoleic acid would represent the Key FAs as it is increasingly present in aquaculture groups of Salema with significantly higher proportions than in urban or wild groups.

On the other hand, there are some inconvenient choosing the FA's profile as bio-indicator of aquaculture influence. For example, even if the amount of linoleic and α-linolenic acids may provide strong signals for measuring the influence of fish farming, however their origin couldn't exclusively be labelled as having been derived from food pellets but could be found in natural marine food, although at low levels (Fernandez-Jover et al., 2011) which follow also our results as we found that most of FA's from terrestrial origin increasingly present in aquaculture group of Salema were equally present in urban group, such as Gamma linolenic acid (18:3n-6), Palmitoleic Acid (16:1n-7) and Alpha linolenic

acid (18:3n-3). In another recent study with Boga, *Boops boops*, Ramirez et al. (in press) have showed that fish near sewage outlets had similar FA profile than those coming from the sea cages. Which means that feed pellets lost from aquaculture cages are not the only source of entrance of terrestrial FA's that could be also shared by waste water release.

Even if from our results, Salemas associated to aquaculture cages present a different feeding behavior compared to wild species which was reflected in its fatty acid profile. It has been widely studied that aquaculture wastes are important inputs of these "terrestrial" fatty acids, other human activities increase the presence of these fatty acids in the marine environment, through sewages and agriculture activities (Quemeneur & Marty, 1992; Seguel et al., 2001). Although, we cannot assume that this changing effect is only attributed to the aquaculture activity as in our results the urban species of Salemas showed also a certain change in their fatty acids profile. Indeed, sewage outfall is a variable source of different fatty acids that directly change the fatty acid profiles of organisms living around them (Yip, 2006). For instance, urban waste waters have been shown to alter fatty acid profile of sediments and marine organisms (Yip, 2006; Dunn et al., 2008). Wong et al. (2008) found a relationship of trophic linkage between mussel fatty acids (*Perna viridis*) and fatty acid profile from suspended particulate matter, affected by domestic sewage.

Conclusion

1. Fish species associated to aquaculture cages had the thinnest otolith compared to wild or sewage outfall-associated communities, probably due to the faster growth rate of fish associated to sea cages. There was no difference in otolith weight between wild and sewage outfall-associated fish.
2. Otolith weigh is very much influenced by feeding behavior and growth rate then we deduced that fish species associated to urban sewage outlet does not show changes in their feeding regime but only an augmentation of nutrients naturally present in the environment. As a result it seems reasonable to consider the study of otolith weigh as a possible indicator of the impact of aquaculture farms.
3. The study performed on otolith shape proved that this parameter is defined either by genetic or environmental factors, therefore invalidating otolith shape as good biomarker for aquaculture impact. Shape otolith seems to vary only after life history segregation between fish population of the same specie.
4. The Geomorphometrical results showed that there was a certain difference in the body shape among the aquaculture groups with respect to wild and sewage outfalls groups. These differences have been localized in the head region, where it appears that the cephalic region from aquaculture groups is reduced compared to wild and urban groups.
5. The analysis of body shape using the GMM technique was considered in this study as an efficient biomarker of the impact of aquaculture in the environment.
6. The geomorphological results from scales analysis did not show any significant result that discriminate among the different groups. The high variability within each group of species invalidates this biomarker to be used as aquaculture-related biomarker.
7. The PCA analysis of muscle fatty acid content did not clearly separate the groups from the different origin, invalidating this parameter as biomarker of aquaculture activity, even when some so-called terrestrial fatty acid increases in aquaculture-related fish. However the increase of ‘terrestrial FAs’ could be due to other environmental factors like the urban and agricultural wastes discharges in the marine waters.

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