Recovery of meagre (*Argyrosomus regius*) population in the Balearic coastal ecosystem (Western Mediterranean).

PhD Thesis

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Recovery of meagre (Argyrosomus regius) population in the Balearic coastal ecosystem (Western Mediterranean) is a Thesis submitted for the degree of PhD by María del Mar Gil Oviedo

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Releasing meagres. (Photo: Jose Mª Valencia)

Abstract

Terrestrial and marine top predators have nowadays become extinct in much of the globe. However, top predators play an important ecologic role because they structure ecosystems and benefit biodiversity. Therefore, management projects are being carried out to recover them. The reintroduction or enhancement of the endangered species into the wild from specimens bred in captivity conditions (hereafter, *restocking*) is one of the most widely used management tools. Nevertheless, restocking has been criticized because it may be neither effective nor economically viable. Although, positive effects have been recorded by increasing the abundance of the population or preserving endangered species.

A responsible approach to marine restocking program involves undertaking pilot studies with tagged fish to assess the effects of restocking and develop strategies that maximize the benefit of restocking and minimize environmental impact. This requires 1) producing a large number of juveniles and 2) releasing and monitoring these fish. Through monitoring of released fish, new knowledge can be obtained on the life history and the ecology of the released species into the wild, which in turn can be used to steadily adapt all the process to the specificities of the target species and the singularities of the area where fish are released.

This PhD project was designed to cover all the relevant issues of a restocking program for the meagre *Argyrosomus regius* in the Balearic Islands coastal ecosystems, ranging from the production of juveniles in captivity to monitoring survival into the wild.

The first requirement to carry out a restocking program is producing in captivity conditions a large number of specimens for release. The spawning of viable eggs by wild meagre broodstock in captivity was obtained by hormonal induction with GnRHa. A specific protocol has been developed for growing fish larvae to juvenile fish consisting in switching at specific ages from rotifers to *Artemia* sp., and finishing with a commercial weaning diet. Then, the juveniles were fed until the optimal size-at-release. For this, a number of diets was compared in terms of growing rate and economic cost. The optimal diet selected for feeding juveniles consists in commercial pellets specifically designed for meagre.

Prior to release, the juveniles were tagged with a double tagging method: an external T-bar anchor tags plus a fluorescent ALR mark at the otolith. This tagging strategy was selected after a number of pilot experiments. Double marking improves retention rate and reduces mortality when compared with other type of tags as internal anchor tags, VIE and OTC. Furthermore, double tagging ensures that released fish can be detected in both short and long term.

This tagging method allowed monitoring of recaptured meagres over time, which in turn has provided the data needed for assessing for first time the movement, diet composition, growth, adaptation and survival of released meagres into the wild.

The movement pattern of released meagres was analyzed using data storage tags, acoustic telemetry and mark recapture data. These combined data showed that meagres remain stationary at shallow sites during daytime and at night they seem to follow a random walk-like type of movement, which results in reaching sites at highly variable depth range (from 0 to 64.3 m) with relatively large and fast displacements. Maximum swimming speed was estimated in 3 km h^{-1}. Therefore, meagre presented a high dispersion capability around Mallorca Island but it is improbable that this species migrates off Mallorca during the juvenile phase.

The diet of released meagres, studied from the stomach content and stable isotopes composition of recaptured meagres, was composed by small decapods during the first months after release but then it changed to a more piscivorous diet consisting in littoral demersal and benthic species.

The growth of the released meagres that spent less than 3 months at liberty was similar or even lower than the expected growth in captivity conditions. Conversely, the growth of meagres that spent more than 3 months was higher than expected. Interestingly, higher than expected size-at-age is related with both, long-term surviving fish were those that already were larger at the release moment, and they experienced an increase in growth rate after releasing.

Differences in the weight of the stomach content, in the body condition index and in the growth showed that released meagres needed a relatively long time (around 3 months) to adapt to the new wild conditions. The released meagres that remain less than 3 months at liberty presented emptier stomach, poorer body condition index and lower or similar growth than control meagres kept at captivity conditions. The meagres that spent more than 3 months tend to present fish in the stomach, they have recovered a good body condition index and they showed higher growth than before release.

The long time needed by released meagres to adapt seems to imply high mortality. When all data were pooled in a single analysis, mortality rate was estimated in 1.69% per day. However, the long term recaptures indicated that this figure probably overestimates mortality. We propose that mortality rate is probably non constant in time, being smaller (by about 0.29% per day) when the released meagres get adapted to the wild conditions.

The reproductive study carried out with meagres in captivity conditions showed that age at maturity (i.e., 50% of probability to be mature) was 2.7 years for males and 3.5 years for females. Therefore, the meagres recaptured after spending two or more than two years at liberty were probably adults. Although there is empirical evidence that some meagres have reached the sexual maturity into the wild, we do not have evidences, as of now, that they have spawned, which would certainly increase the chance of success of the meagre restocking program. Nevertheless, reducing short-term mortality appears to be the most important challenge for increasing the chance of recovering a sustainable population of meagre in the Balearic Islands.

Resumen

Los depredadores apicales, tanto terrestres como marinos, han sido eliminados de la mayor parte de los ecosistemas del mundo. A pesar de esto los depredadores apicales tienen un papel ecológico clave en la estructura del ecosistema y beneficia el mantenimiento de la biodiversidad. Por tanto se están llevando a cabo diversos proyectos encaminados a recuperar estas especies. Una de las medidas de manejo más utilizadas para lograr la reintroducción de especies extinguidas o para mejorar el estatus de poblaciones en peligro consiste en liberar al medio natural individuos criados en cautividad, técnica conocida como *repoblación*. A pesar de eso, la repoblación también ha sido criticado, aduciéndose que puede ser inefectivo o económicamente inviable. Por contra, sus aspectos positivos se han puesto también en evidencia en casos en los que se ha incrementado la abundancia de determinadas poblaciones o se ha preservado con éxito determinadas especies en peligro.

Una aproximación responsable a la repoblación de especies marinas implica desarrollar toda una serie de estudios piloto con individuos marcados para evaluar empíricamente los efectos de la repoblación y minimizar sus posibles impactos ambientales. Esto requiere 1) producir un gran número de juveniles y 2) liberar y monitorizar estos peces. Gracias al seguimiento riguroso de los peces liberados se genera un volumen de información muy importante sobre diferentes aspectos de la historia vital y de la ecología de la especie en cuestión, que a su vez puede ser utilizada para adaptar de manera inmediata todo el proceso de repoblación a las especificidades tanto de la especie como del área donde los juveniles son liberados.

Este proyecto de Tesis Doctoral ha sido diseñado para cubrir todos los aspectos relevantes relacionados con el programa de repoblación de la corvina *Argyrosomus regius* en los ecosistemas costeros de las Islas Baleares, cubriendo desde la producción de juveniles en cautividad hasta el seguimiento de su supervivencia en el medio natural.

El primer requerimiento para llevar a cabo un programa de repoblación es la capacidad de producir en cautividad un elevado número de juveniles. La producción de huevos viables por parte de un stock de individuos reproductores capturados del medio natural ha sido posible gracias a la inducción hormonal con GnRHa. Se ha desarrollado un protocolo especifico para criar las larvas obtenidas hasta la fase de juvenil, consistente en el cambio desde una alimentación inicial a base de rotíferos, a una dieta

de *Artemia* sp., para finalizar con una dieta comercial de destete. A partir de la fase de juvenil, los peces fueron alimentados hasta alcanzar la talla óptima para ser liberados. Para ello, se comparó un amplio abanico de posibles dietas, tanto en términos de crecimiento como de coste económico. La dieta óptima para alimentar juveniles consistió en un granulado comercial específicamente diseñado para corvina.

Antes de ser liberados, los juveniles fueron marcados mediante una marca doble: una marca externa de tipo T y una marca fluorescente de ALR en el otolito. Esta estrategia de marcado fue seleccionada tras una serie de experimentos piloto. El doble marcado mejora la tasa de retención y reduce la mortalidad, al compararse con otros tipos de marcas como las marcas internas de tipo ancla, VIE o OTC. Además, el doble marcado asegura que los peces liberados puedan ser identificados tanto a corto como a largo plazo.

Este método de marcado ha permitido un seguimiento de las capturas de corvina a lo largo del tiempo y el análisis de dichas capturas han permitido a su vez obtener por primera vez datos acerca del movimiento, la dieta, el crecimiento y la supervivencia de los peces liberados al medio natural.

El patrón de movimiento de las corvinas liberadas fue analizado mediante marcas de toma de datos, telemetría acústica y datos de captura/recaptura. Tras combinando las tres fuentes de información, se demuestra que la corvina permanece estacionaria y a poca profundidad durante el día, mientras que durante la noche parece moverse siguiendo algún tipo de desplazamiento aleatorio (*random walk*), caracterizado por desplazamientos relativamente largos y rápidos. La velocidad máxima fue estimada en 3 km h^{-1}. Por tanto, la corvina presenta una capacidad de dispersión muy elevada alrededor de la costa de Mallorca pero es improbable que esta especie sea capaz de migrar fuera de Mallorca durante la fase juvenil.

La dieta de las corvinas, estudiada mediante el contenido estomacal y la composición de isótopos estables en varios tejidos de los peces recapturados, está compuesta por decádopos de pequeña talla durante los primeros meses después de ser liberadas, pero a partir de cierto tiempo pasa a ser más piscívora, con peces demersales y bentónicos.

El crecimiento de las corvinas liberadas que han pasado menos de tres meses en libertad fue similar al crecimiento esperado en cautividad. Contrariamente, el crecimiento de las corvinas liberadas que han pasado menos de tres meses en libertad es mayor que el esperado. Es muy interesante destacar que tener una talla esperada a una edad dada mayor de lo esperado, está relacionado tanto con una mayor supervivencia a largo plazo de los peces que ya eran mayores en el momento de ser liberados, como con un incremento en la tasa de crecimiento después de la liberación.

Las diferencias en el peso del contenido estomacal, el índice de condición y en el crecimiento demuestran que las corvinas necesitan un periodo de adaptación al medio natural relativamente largo (unos tres meses). Las corvinas liberadas que pasan menos de tres meses en libertad presentan estómagos más vacíos y peores índices de condición al compararlas con corvinas control que han permanecido todo el tiempo en cautividad. Las corvinas que pasan más de tres meses en libertad, recuperan un buen índice de condición y presentan mejores tasas de crecimiento que antes de ser liberadas.

El dilatado periodo de tiempo necesario para que las corvinas liberadas se adapten al medio natural implica una elevada mortalidad. Cuando todo los datos fueron agrupados, la tasa de mortalidad se estimó en 1.69% por día, pero el numero esperado de recapturas a largo plazo cuando se utiliza esta tasa es mucho menor que el observado, sugiriendo que al agrupar todos los datos se sobreestima la mortalidad. Se propone, por tanto, que la tasa de mortalidad no es constante en el tiempo, diferenciándose una primera etapa, justo después de la liberación, con una moralidad muy elevada y una segunda etapa con mortalidad mucho más reducida (estimada en aproximadamente 0.29% por día), a partir del momento en que las corvinas se adaptan a las condiciones del medio natural.

El estudio de las características reproductivas que se ha llevado a cabo con las corvinas en cautividad muestra que la edad de maduración (edad a la que el 50% de la población es sexualmente madura) es de 2.7 años en el caso de los machos y de 3.5 años para las hembras. Por tanto, los peces recapturados al cabo de unos dos años después de la liberación son ya probablemente adultos. A pesar de que se han obtenido evidencias empíricas de que al menos algunas corvinas han alcanzado la madurez sexual en el medio natural, no se tienen evidencias de que estos ejemplares se hayan reproducido, siendo esto último un muy buen indicio de las posibilidades de éxito del programa de repoblación de la corvina. En cualquier caso, reducir la mortalidad a corto plazo parece ser el reto más importante a la hora de incrementar las posibilidades de éxito de recuperar una población sostenible de corvinas en las Islas Baleares.

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Meagre broodstock in LIMIA’s sea cage. (Photo: Reuters/Enrique Calvo)

Introduction

The meagre, *Argyrosomus regius* (Asso, 1801), is a top predator and one of the world's largest sciaenid fish that is widely distributed along the eastern Atlantic coast (from Norway to Congo), including the Mediterranean (Chao, 1986). It's a semi-pelagic coastal species with demersal trends (Quéro & Vayne, 1987) that inhabits sandy bottoms, in which can be found both close to the bottom as well as near-surface and in midwaters, within a depth range between 15 and 300 m (Schneider, 1990). Because of its distribution, large size, high ex-vessel prices, and high seasonal availability in inshore and nearshore waters, the meagre constitutes an important target species for many local small-scale multi-gear multi-species commercial fleets, purse seiners, bottom trawlers and the recreational sector (Quéro & Vayne, 1987; Quéméner, 2002; Morales-Nin *et al.*, 2010a).

Since the twenties, and due to the increasing interest in the meagre as a candidate in marine aquaculture diversification, the somatic growth, feeding and larval survival of reared meagres have been described (Piccolo *et al.*, 2008; Chatzifotis *et al.*, 2010; Roo *et al.*, 2010; Estévez *et al.*, 2011) and some aspects of its life-history have been studied (Morales-Nin *et al.*, 2010a; González-Quirós *et al.*, 2011; Morales-Nin *et al.*, 2012). However, there is a lack of basic biological information on which to base management rules. They are still many gaps in what regards the biology, reproduction, ecology, and population dynamics of the species.

A priori, meagre appears to be a resilient species due to fast growth (Sadovy & Cheung, 2003) and high fecundity (Gil *et al.*, 2013). However, due to its inherent biological vulnerability, together with overfishing and environmental degradation of spawning habitats, the abundance of meagre has decreased alarmingly (Quéro & Vayne, 1987; Sadovy & Cheung, 2003). Nowadays, this species is considered extinct in the Balearic Islands (western Mediterranean), where it was a relatively frequent capture only a few decades ago (Mayol *et al.*, 2000).

Yield reduction in fish resources has stimulated the development of many management strategies. Among others, there is an increasing interest in using hatchery-reared animals to enhance wild stocks (*restocking*, hereafter) (Cowx, 1994; Munro & Bell, 1997; Bartley & Bell, 2008). Recently, meagre has been shown to be a feasible candidate for the diversification of finfish marine aquaculture (Quéméner, 2002; Jiménez *et al.*, 2005) and the development of meagre aquaculture techniques has provided meagre reared juveniles (Quéméner, 2002; Pastor & Grau, 2013) to carry out a restocking program.

In this Chapter, an overview of the current knowledge about the biology and ecology of the meagre is presented. Then, the status of the meagre populations is described and the restocking as a management tool to recover depleted or endangered populations is analyzed. Finally, the objectives and the organization of this Thesis are outlined.

1.1 The meagre: Argyrosomus regius

1.1.1 Taxonomy

The meagre, *Argyrosomus regius*, is a Teleost species belonging to the family Sciaenidae within the Order Percomorphi (Perciformes), suborder Percoidei. This family includes about 70 genera and 270 marine, brackish and fresh water species distributed all over the world (Chao, 1986; Nelson, 2006). There are three genera and five species of the family present in the Northeast Atlantic and the Mediterranean (Quéméner, 2002):

Sciaena

Sciaena umbra Linnaeus, 1758; escorball

Umbrina

Umbrina canariensis Valenciennes, 1843; not present at the Balearic Islands

Umbrina cirrosa Linnaeus, 1758; reig

Umbrina ronchus Valenciennes, 1843; not present at the Balearic Islands

Argyrosomus

Argyrosomus regius Asso, 1801; corbina, corb reig o corball blanc

According to Fishbase (July 2013; http://www.fishbase.org), the genus *Argyrosomus* comprises 9 species (Table 1.1).

No.	Valid Name	Author	English Name
1.	*Argyrosomus amoyensis*	Bleeker, 1863	Amoy croaker
2.	*Argyrosomus beccus*	Sasaki, 1994	
3.	*Argyrosomus coronus*	Griffiths & Heemstra, 1995	Dusky kob
4.	*Argyrosomus heinii*	Steindachner, 1902	Arabian sea meagre
5.	*Argyrosomus hololepidotus*	Lacepède, 1801	Southern meagre
6.	*Argyrosomus inodorus*	Griffiths & Heemstra, 1995	Mild meagre
7.	*Argyrosomus japonicus*	Temminck & Schlegel, 1843	Japanese meagre
8.	*Argyrosomus regius*	Asso, 1801	Meagre
9.	*Argyrosomus thorpei*	Smith, 1977	Squaretail kob

Table 1.1.- Species with the genus Argyrosomus. Source: Fishbase.

The genus *Argyrosomus* was defined by De la Pylaie (1835). This genus is characterised by large fishes with elongate body and with 3-5 small upper pores present on tip of snout and chin without barbel, but with three pairs of mental pores. Caudal fin truncates to slightly S-shaped in adults. The swimbladder is carrot-shaped, with 36-42 pairs of complicated arborescent appendages, unlike *Umbrina* and *Sciaena,* with a simple swimbladder. A feature typical of the family is the presence of large, oval and thick otoliths (*sagittae*), to which medical properties were attributed in the past (Duffin, 2007), with a tadpole-shaped sulcus on its inner surface, which in the case of *Argyrosomus* presents a granular outer surface (Fig 1.1).

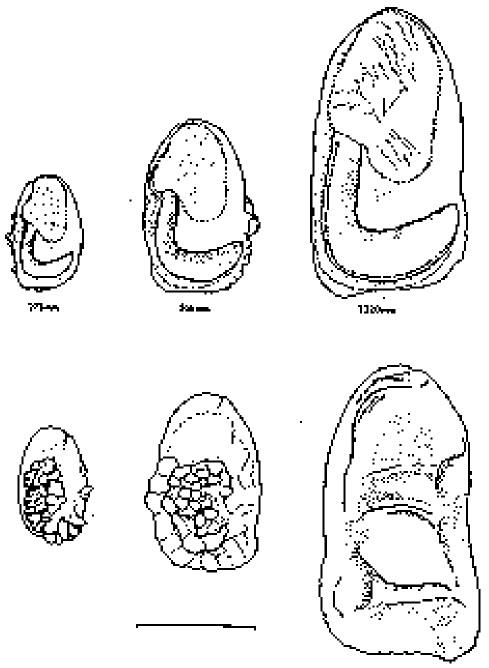


Fig. 1.1.- Proximal side (above) and distal side (below) views of sagittae of *Argyrosomus regius*. Scale bar = 10 mm. Source: Griffiths & Heemstra, 1995. Ichthyological Bulletin.

The only member of the genus *Argyrosomus* present at the Balearic Islands is *Argyrosomus regius* (Asso, 1801). This species was first described by the Spanish naturalist Ignacio Jordán de Asso y del Río (1801) under the name of *Perca regia*, inhabiting the waters of the eastern Spanish coast. The species was later assigned to the genus *Argyrosomus* described by De la Pylaie (1835).

1.1.2 Morphology

Argyrosomus regius presents a nearly fusiform, elongated and slender body, slightly compressed laterally. It resembles a European seabass (*Dicentrarchus labrax*) in form, but with pearly-silver coloration, darker on back, with bronze reflections on sides, fin base reddish brown and a yellow-coloured mouth when fresh (Fig. 1.2; Chao, 1986; Quéméner, 2002; FAO, 2005-2013). Post-mortem colour is brown. It's a large fish, and can reach up to 200 cm total length (L_T) with weights up to 55 kg, and a maximum age of 42 years (Quéro & Vayne, 1987; González-Quirós *et al.*, 2011). The largest specimen of *A. regius*, as recorded in scientific literature, was of 230 cm L_T (Maigret & Ly, 1986) and the maximum published weight was 103 kg (Quéro & Vayne, 1987).

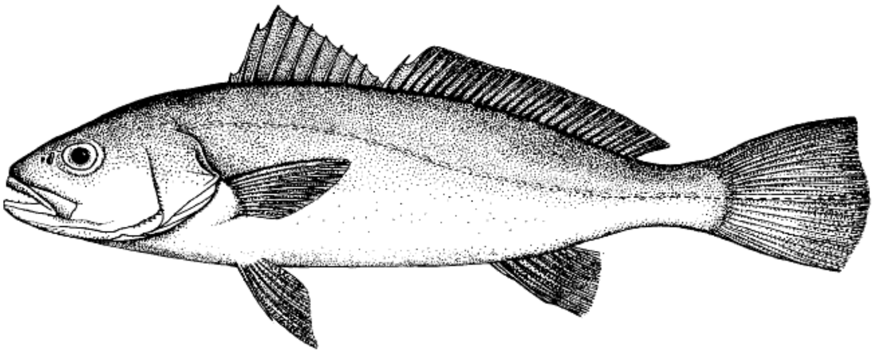


Fig. 1.2.- The meagre, *Argyrosomus regius* (Asso, 1801). Source: Mayol *et al.*, 2000. Llista Vermella dels Peixos de les Balears. Govern de les Illes Balears.

The head is relatively big with a large, oblique and terminal mouth without barbils. Inside the mouth there are narrow bands of villiform teeth in the upper jaw with the outer row enlarged. In the lower jaw, teeth form 2 or 3 irregular rows, one of them enlarged. The eyes are quite small. The lateral line is evident, extending onto caudal fin. The second dorsal fin is much longer than the first one. The first dorsal fin has 9-10 spines and the second dorsal fin has 1 spine and 26-29 soft rays. Anal fin has a first short spiny ray and a second very thin one with 7-8 soft rays afterwards. Caudal fin is truncated to S-shaped. The body is covered with very big ctenoid scales, except for some cycloid scales on chest, snout and below eyes. 36-42 arborescent branched appendices are present in the gas bladder, with sonic muscles, bigger in males, in close association with the swim-bladder which can vibrate producing two distinct sounds: a typical and regular long 'grunts' and sometimes also short grunts (FAO, 2005-2013; Lagardère & Mariani, 2006). Very large otoliths (*sagittae*) are located inside the otic capsules, at the base of the skull, underneath the pharyngeal teeth and near the dorsal insertions of the first gill arches. These otoliths continue to be used as amulets by fishermen in the south of the Iberian Peninsula (Cárdenas, 2010). Meagre *sagittae* presents distinct morphological features on its proximal (or inner) and distal (or outer) sides. The most conspicuous features are a tadpole shaped *sulcus acusticus* on the proximal side (further divided into an anterior *ostium* and a posterior *cauda*) and a conspicuous protuberance termed “umbo” on the distal side (Prista *et al.*, 2009).

1.1.3 Distribution, habitat, migration and reproduction

The meagre is widely distributed along the eastern Atlantic coast, northward to English Channel, North Sea and southern Norway and Sweden, including a single record from Iceland; southward to Congo, including the Canaries. Also it is found in the whole Mediterranean, in the western end of Black Sea and in the Sea of Marmara, in lakes of Nile Delta and in Bitter Lakes to Gulf of Suez (Chao, 1986). Indeed, this species has also been cited in the northern Red Sea due to an Anti-Lessepssian migration via the Suez Canal (Steinitz, 1967).

The species form large reproductive spawning aggregations in certain localities, well known by the fishermen. In the Eastern Atlantic, the species specially congregates, northwards, at the Gironde estuary (Quéro & Vayne, 1987; Quéro, 1989), and at the mouths of the rivers Guadiana (Prista *et al.*, 2007) and Guadalquivir (Sobrino *et al.*, 2005; Catalán *et al.*, 2006a). Southwards, another very important spawning area is located at the Lévrier Bay, in Mauritany (Quéméner, 2002). In the Eastern Mediterranean, the main confirmed spawning area is located at the mouth of the Nile river and related areas (Lakes of Nile delta; Quéro, 1985). However, the species has been strongly rarified in the western Mediterranean (Quéro, 1985). It’s noteworthy that the main reproductive areas of the species are located within or near estuaries and river mouths with brackish waters, except in the case of the Lévrier Bay in Mauritany, where the salinity of the waters is higher than average of the zone (Tixerant, 1974). The distance between these main breeding areas seems to indicate the existence of isolated populations (Sourget & Biais, 2009). In fact, it has been hypothesized that the Mauritany population may be different from the Gironde-Gascogne population, as seems to show the different form of the otoliths of the fishes from these two localities (Tixerant, 1974).

It's a eurihaline, semi-pelagic coastal species with demersal trends (benthopelagic; Quéro & Vayne, 1987) inhabiting inshore and shelf waters, preferentially on muddy or sandy bottoms (Quéméner, 2002) and occasionally on rocky bottoms (Cárdenas, 2010). It can be found close to the bottom as well as near-surface and midwaters, from 15 to 300 m (Schneider, 1990). Juveniles and sub-adults are common in estuarine and shallow coastal areas, forming schools (Chao, 1986; Quéro & Vayne, 1987; Quéro, 1989; Quéméner, 2002; Catalán *et al.*, 2006a). Adults are solitary or in small groups, moving offshore into deep-waters during the non-reproductive season (Morales-Nin *et al.*, 2012), but during the spawning season meagre migrates inshore and congregates to spawn near estuaries and coastal areas (Quéro & Vayne, 1987; Griffiths & Heemstra, 1995; Quéméner, 2002; Cárdenas, 2010; Pastor & Grau, 2013) in very large spawning aggregations producing conspicuous sounds (Quéro & Vayne, 1987; Griffiths & Heemstra, 1995; Quéméner, 2002; Lagardère & Mariani, 2006). During these reproductive aggregations, meagre, especially the males, produces two distinct sounds, later in the afternoon: regular long grunts, the most common calls, that is suggested to serve the formation of spawning aggregations, and short grunts, that is suggested to announce the beginning of the courtship behaviour (Lagardère & Mariani, 2006). This is in accordance with the evening spawning proposed for all the sciaenids by Holt et al. (1985), in order to reduce predation on sciaenid eggs by allowing dispersal of eggs during the night when planktivores may be less active.

Meagre is considered a subtropical, oceanodromous species, performing both adult and juvenile migration movements, in distance and depth, linked to reproduction in the case of adults and to feeding in the case of juveniles, along shore or offshore-onshore in response to temperature change (Quéro, 1989; Griffiths & Heemstra, 1995). Water temperature seems to be the determining factor in the trophic and reproductive migrations (Quéro, 1989). In fact, there is a pattern of movement described in the Gironde estuary: adults entry to spawn in May and juveniles (age classes 0, 1 and 2) exit from the Gironde estuary in October, both movements occur when the water temperature reaches 13-14 ºC (Quéro, 1989). After spawning, adults leave the Gironde estuary from mid-June to late July, to feed along the shore until the fall. In autumn, they seek deeper waters, probably inside the south trenches of Gascogne, to spend the winter (Quero & Vayne, 1993). On the other hand, juveniles leave the Gironde estuary in the autumn and form schools in the coastal waters (20-40 m) between Arcachon and Charentais to spend the winter. In mid-May, they return to the feeding-rich waters of the Gironde estuary to nourish (Quéro & Vayne, 1987). The raise of temperature to 17-20 ºC marks the onset of spawning (Quéro & Vayne, 1987; Pastor & Grau, 2013).

This reproductive migration hypothesis is consistent with the complex conceptual model of life history migrations recently proposed for this species in the Gulf of Cádiz (González-Quirós *et al.*, 2011; Fig. 1.3) and confirmed by otolith geochemical signature studies (Morales-Nin *et al.*, 2012), although differences in the reproductive period, growth rate, and size or age-at-first-maturity may occur as a consequence of differences in oceanographic and ecosystem characteristics and fishing pressure.

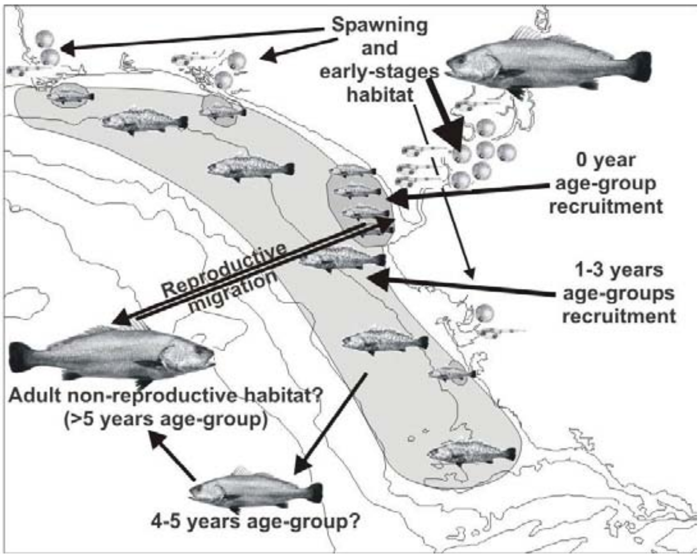


Fig. 1.3.- Migratory meagre behaviour based on the fishing and biological data. Source: González-Quirós *et al.*, 2011.Fisheries Research.

Namely, in the Gulf of Cádiz, large-size adults (age-at-maturity probably occurring in males at 5 years of age and 6.6 years in females; Morales-Nin *et al.*, 2012) approach estuarine and salt-marshes from March to August to reproduce (González-Quirós *et al.*, 2011), those being also the habitat of larval and early-juvenile stages (Catalán *et al.*, 2006a). Thereafter, juveniles (*ca.* 7-25 cm L_T) recruit in open waters nearby the

Guadalquivir mouth and along the coast (5-10 m depth contour) throughout the year (Catalán *et al.*, 2006b). Premature meagres (*ca*. 20-70 cm L_T and 0^+-3 years) may progressively expand their habitat and are fished all year round by local fleets in coastal waters of the Gulf. Most meagres spend the first 2-4 years in offshore waters (Morales-Nin et al., 2012). Meagres above 3 years old and at a size around 70 cm L_T are underrepresented in the landings of local fleets, until they mature and then migrate back to estuaries and salt-marshes to reproduce every year from March to August. Out of the reproductive season adults are underrepresented in the catches of local fleets (González-Quirós *et al.*, 2011).

Meagre is believed to be an iteroparous, monomorphic, gonochoristic species with ovarian asynchronous development (García-Pacheco & Bruzón, 2009). The only estimates of length-at-maturity described at the literature were 61.6 cm in males and within the 70-110 cm range in females at the Cádiz population (González-Quirós *et al.*, 2011), 60 cm in males and above 80 cm in females at the Gascogne Gulf population (Sourget & Biais, 2009), and above 72 cm in males and above 82 cm in females at the Mauritany population (Tixerant, 1974). The spawning period of meagre, which is highly plastic, varies from 15 days at the Gironde estuary (at the beginning of June) to 8-9 month in Mauritany (from October to June; Quéro, 1989). In the Gulf of Cádiz, the spawning period comprises 5 months, from March to August (González-Quirós *et al.*, 2011). In the mouth of the Nile River, spawning occurs from October to December, comprising 3 months (Quéro, 1985).

1.1.4 Diet

Meagre is a voracious fish which has the ability to feed on many prey groups and species (Quéro, 1985; Quéro & Vayne, 1987; Cabral & Ohmert, 2001; Jiménez *et al.*, 2005; Baldó *et al.*, 2006; Pasquaud *et al.*, 2008; Pasquaud *et al.*, 2010). In general, the diet of meagre consists of fish and swimming crustaceans (Chao, 1986). However, the proportion of these preys in the diet varies according to the size of the fish. Juvenile meagre has low prey diversity, diet mainly consisting on crustaceans like Mysidacea, Isopoda, Amphipoda and Decapoda (Quéro & Vayne, 1987; Baldó & Drake, 2002; Jiménez *et al.*, 2005). Mysidacea and the brown shrimp *Crangon crangon* are the major prey items in wild juveniles in the Tagus estuary (Cabral & Ohmert, 2001). Later, meagre gradually shifts their main preys from decapods to fish with size (Jiménez *et al.*, 2005). The fish consumed by meagre are mainly pelagic species such as clupeids (mainly the European anchovy *Engraulis encrasicolus*, and pilchard *Sardina pilchardus*), mugilids, and scombrids (like the mackerel *Scomber scombrus*) but benthic fish has also been described, such as Gobiidae and Soleidae (Quéro & Vayne, 1987; Pasquaud *et al.*, 2008; Pasquaud *et al.*, 2010). The knowledge of the fishermen says that meagre enters in the fisheries pursuing shoals of clupeids and mugilids (Chao, 1986; Quéro & Vayne, 1987). For that reason, it has been hypothesized a predator-prey relationship between cyclical migrations of sardines (offshore-inshore in spring, inshore-offshore in winter) and migrations of meagres (Quéro & Vayne, 1987).

1.2 Status of meagre populations

1.2.1 Economic interest

1.2.1.1 Fisheries

This sciaenid is a highly prized species that is targeted by recreational and commercial fleets (Morales-Nin *et al.*, 2010a). For that reason, global world catches of meagre have sharply increased since 1950 (FAO, 2000), being dominated during the 50s by the landings in the North Eastern Atlantic (Biscay Bay Fishery, Spain) but, from 1961 onwards, there has been a sharp decline in catches in that area. In 1998, the total annual catch was 9175 tonnes (FAO, 2000), capturing Spain less than 1% of world production. Average world catches of meagre were 4752 t year^{-1} from 2005 to 2010 (Fig.1.4; FAO, 2012b), but the real volume of landings is most likely greater because the FAO data for some countries are likely to be underestimated (González-Quirós *et al.*, 2011). Nowadays, its largest fisheries are located in the Central Eastern Atlantic (Mauritany and Morocco) and Egypt, which together suppose over 80% of the total world annual catch, estimated in about 6000 t (FAO, 2012b). Mauritany, with 65% of the total world annual catches, is the main producing country. It is important to note that there are huge fluctuations in annual catches in all fisheries of the species (Quéméner, 2002). For example, in the Mediterranean, Egypt is practically the only producing country, with an annual average of 800 t since 1989, but with extreme fluctuations varying from 72 t in 1972 to 2324 t in 2004 (FAO, 2000; FAO, 2012).

In European countries, annual meagre landings are generally below 1500 t and the fish is of secondary importance in national capture production totals (FAO, 2012b), except in France where the landings at the fishery of the Gascogne-Gironde Gulf (North Eastern Atlantic) have sharply increased since 1996, with periodic cyclical fluctuations of about 7-8 years. Now, they are estimated in 700-1400 t from year 2005 onwards, being mainly on juveniles less than 2 kg, but there were practically nulls in the 80s (Sourget & Biais, 2009).

In Spain, this species is mainly captured by the artisanal (small-scale) fleet, purse-seiners and bottom trawlers and the recreational sector. There is only one active commercial fishery, which is located at Cádiz Bay (SW Spain). The average landings are 159 t year^{-1} (González-Quirós *et al.*, 2011). Also, there are some landings at the Biscay Bay, where 90% of current commercial catches are below 2 kg, as in the neighboring region of the Gascogne-Gironde Gulf. Commercial fishing is practiced mainly with purse-seines in the breeding season, when the fish are grouped in large spawning aggregations (see Chapter 1.3), but the species is also captured with trammel nets and longlines throughout the year. Bottom trawlers usually captured juveniles nearshore at the mouth of the Guadalquivir river. The recreational sector mainly caught meagres by trolling using live bait (squid) or employing artificial lures that simulate a small squid, or by spearfishing in rocky bottoms between 15 and 30 m depth (Pastor & Grau, 2013). But the main landings in that fishery occur when meagre becomes available to the fishery when they approach the estuaries and salt marshes to reproduce (see Chapter 1.3), then overfishing is likely to occur there (González-Quirós *et al.*, 2011).

1.2.1.2 Aquaculture

In the last year meagre has become a feasible candidate for the diversification of finfish marine aquaculture (Quéméner, 2002; Jiménez *et al.*, 2005) and its production has increased in the last years, reaching values higher than those obtained by fishing catches and highlighting the appearance of a new aquaculture species on the market (Fig. 1.4; Monfort, 2010; FAO, 2012b, 2012a). Meagre culture started in France and in Italy in the late ‘90s, whereas Spain entered into the business in 2004, followed in 2007 by Greece and Turkey (Monfort, 2010). Egyptian production started later on. The total aquaculture production has jumped from a few tonnes in 2000 to around 4000 t in 2008 (FAO, 2012a). However, nowadays, Egyptian production has become the most important, reaching 12246 t in 2010. Although Spain is the second largest producer of meagre only reached 1853 tons during the same year (FAO, 2012a) and the average fish market price was 4 euros, beating the average price of sea bream (3.75 euros). Before long, the meagre has become the fourth most important species in the Spanish marine aquaculture fish production behind sea bream, sea bass and turbot (Pastor & Grau, 2013).

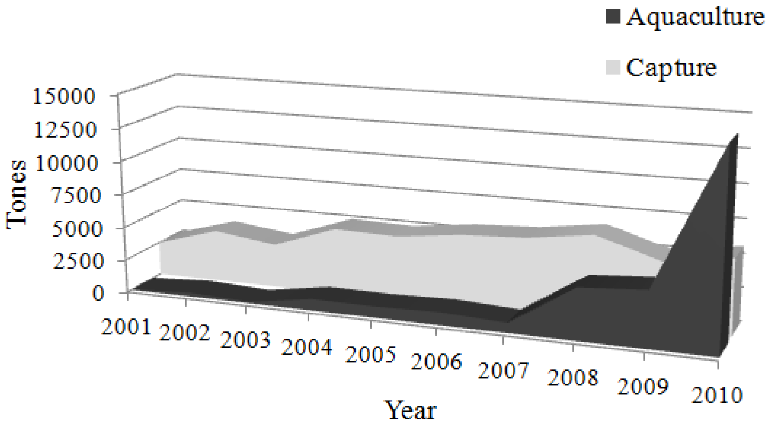


Fig. 1.4.- Evolution of world meagre fishing catches and aquaculture production. Source: FAO statistics.

1.2.2 Vulnerability of sciaenids

A priori, meagre appears to be a resilient species due to fast (Sadovy & Cheung, 2003) and high fecundity (Gil *et al.*, 2013). However, nowadays, meagre, as many others sciaenids, is considered a highly vulnerable species due to its inherent biological vulnerability (Sadovy & Cheung, 2003). The main reasons of this high biological vulnerability are its large size, longevity and late age-at-maturity coupled with its reproductive behaviour (large spawning aggregations and sound production make meagre easily found and captured, see Chapter 1.3) and the often serious environmental degradation of the spawning habitats (Quéro & Vayne, 1987; Quéro, 1989; Baldó & Drake, 2002; Catalán *et al.*, 2006a; Lagardère & Mariani, 2006; González-Quirós *et al.*, 2011; Morales-Nin *et al.*, 2012). For many of these reasons, together with high ex-vessel prices which make them commercially prized species, there is a general worldwide tendency to overfish top predators (Christensen *et al.*, 2003), sciaenid among others including meagre (Quéro and Vayne, 1987; Piner and Jones, 2004; Silberschneider et al., 2009).

In Mediterranean waters, the abundance of meagre has decreased alarmingly (Quéro & Vayne, 1987; Sadovy & Cheung, 2003), as well as in other North Eastern Atlantic areas (Dulvy *et al.*, 2003). An example of this is the huge yield fluctuations at the main fishery Mediterranean area, the Nile Fishery (see Chapter 2.1.1). Some explanations of these oscillations have been hypothesized: the increased halieutic exploitation, together with the construction of the Aswan Dam, which has changed the hydraulic regime of the Nile, explains the production decline detected in Egypt (Bebars *et al.*, 1997). The increased salinity recorded in the breeding areas in the Nile Delta can also justify the decline in production (EL-Hehyawi, 1974). Moreover, the Lessepssian migrant called the narrow-barred Spanish mackerel *Scomberomorous commerson*, which uses a similar niche as the meagre (both species are piscivorous), may have moved the indigenous meagre from Israelian coasts (Nile Fisheries) causing the complete disappearance of the species in local catches where it was once one of the most common commercial species (Golani, 1998).

At present, this species is considered extinct in the Balearic Islands (western Mediterranean), where it was a relatively frequent capture only a few decades ago (Riera *et al.*, 1997; Mayol *et al.*, 2000). Last citations of its presence at the Balearic Islands, where it has been cited since 1775 by Vilella in the "*Catálogo de especies enviadas al Museo de Ciencias Naturales*" (Azcárate, 1990), are from 1962 and 1974-1975 (Mayol *et al.*, 2000). Moreover, there are not records now of landings of wild meagres in all the fishing ports of the Spanish Mediterranean coastline (Pastor & Grau, 2013) nor there are reports of the observation of large spawning aggregations of the species in all the western Mediterranean (Quéro, 1985).

The above information raises concern regarding the status of meagre stocks worldwide, exacerbated by the lack of basic biological information on which to base management rules. For example, it is not listed on the Red List of endangered species by the International Union for Conservation of Nature (IUCN) because the available data are too scarce. Moreover, knowledge regarding other aspects that are related to meagre life history is insufficient and fragmentary. But all signs seem to point that the meagre, *Argyrosomus regius*, is overexploited at all the world's fisheries worldwide.

1.3 The restocking programs as a management tool

1.3.1 Background

Marine fish populations are declining worldwide (Blankenship & Leber, 1995), due to environmental degradation and inappropriate fisheries management have caused almost all fisheries to decline and even to collapse (Born *et al.*, 2004). The continuous increase in human population size worldwide suggests this trend will continue into the future (FAO, 2012b). Three conventional tactics have been considered to recover depleted stocks and manage fisheries: restoring degraded nursery and spawning habitats, regulating the intensity of fishing and releasing cultured fish (i.e., restocking) (Blankenship & Leber, 1995).

Restocking is not a new concept, dates from the middle of the 19th century, when hatched salmonids were released into rivers in Sweden, the USA and Germany (Svåsand *et al.*, 2000). Later, stocking of marine species (e.g., cod in Norway; Sars in 1879) was carried out too (Svåsand *et al.*, 2000). Currently, there are nearly 70 countries with active marine and coastal stocking programs, ranging from experimental research or large scale industrial releases (Fig. 1.5). However, the real number could be higher because some countries that have marine stocking programs do not report these in scientific literature (Born *et al.*, 2004).

Approximately 180 different species being released of which 46 species are confined to marine environments (Born *et al.*, 2004). More than ten different marine and coastal species are stocked in the United States, Republic of Korea (North Korea), Australia, Japan, France and Poland. For example, in Japan over 80 species are reported to be reared for stock enhancement (Masuda & Tsukamoto, 1998). The major species produced for restocking programs are shown in Table 1.2.

Species	Total no. produced between 1984-1997	Average no. of years reported	No. of countries	Countries
Patinopecten yessoensis	5656216000	3	1	Japan
Pagrus major, Acanthopgrus schlegeli, Ebynnis japonica	105019000	2	1	Japan
Paralichthys olivaceus	55878000	2	1	Japan
Tridacna maxima	25020000	1	1	Tonga
Penaeus vannamei, P. stylirostris	22500000	1	1	Panama
Pagrus major	14230000	3	3	Japan, Republic of Korea, Taiwan Province of China
Seriola quinqueradiata, S. aureovitlata, S. durmerilli	13963000	3	1	Japan
Portunus trituberculatus	9361000	7	1	Republic of Korea
Strombus gigas	5700000	1	3	Belize, Sao Tome, Turks & Caicos Islands
Paralichthys olivaceus	3593000	5	2	Japan, Republic of Korea
Sebastes schlegeli	2739000	7	1	Republic of Korea
Acanthopagrus schlegeli	1009000	9	1	Republic of Korea
Haliotis diversicolor aguatilis	889000	2	1	Taiwan Province of China
Oplegnathus fasciatus	613000	6	1	Republic of Korea
Penaeus (orientalis) chinensis	1370000000	1	1	China
Penaeus japonicus	366327501	3	4	Japan, Republic of Korea, Portugal, Taiwan (China)
Scallop	165937000	3	1	USSR
Crassostrea gigas	160140000	3	1	Japan
Penaeus orentalis kishimouye	106655000	8	1	Republic of Korea
Penaeus monodon	26316000	1	5	Brunei, Republic of Korea, Mauritius, Seychelles
Penaeus merguiensis	16925800	1	1	Malaysia
Mugil incillis	15468000	3	1	Colombia
Sciaenops ocellatus	14100045	3	1	USA
Sparus auratus	7620000	1	3	Cyprus, Egypt, Portugal
Penaeus stylirostris	1500000	1	1	Panama
Sparus auratus, Dicentrarchus labrax	950000	1	1	Cyprus

Table 1.2.- Major species in terms of hatchery production for release into natural environments. Source: Born et al., 2004. Marine Ranching. FAO.

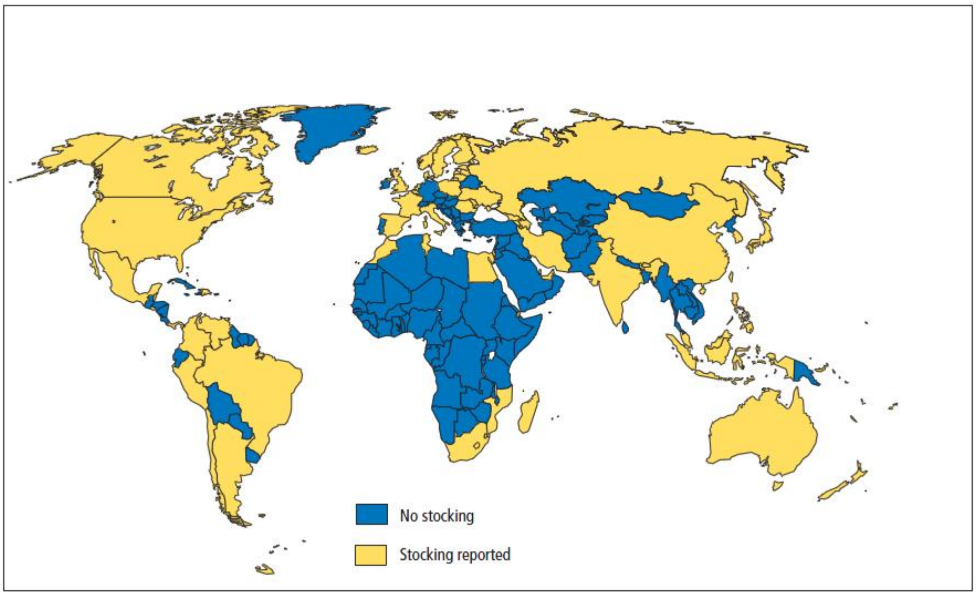


Fig. 1.5.- Countries with reported marine restocking. Source: Born et al., 2004. Marine Ranching. FAO.

1.3.2 Pros and cons of the restocking programs

Restocking has been criticized because it may be neither effective nor economically viable. Limited usefulness of stocking has been attributed to the fact that cultured fish have tended to show lower survival, growth rate, reproductive fitness and genetic diversity than have wild fish (Bartley, 1996; Araki & Schmid, 2010). Additional adverse consequences of releasing cultured fish are ecological competition with wild fish, genetic introgression or diseases (Frankham *et al.*, 2002; Bell *et al.*, 2008; Kostow, 2009; Lorenzen *et al.*, 2012).

However, positive effects have been widely evidenced. For example, the recovery of red drum stocks in Texas through the initiation of an overall recovery plan including stocking and fisheries regulations (McCarty *et al.*, 1993). Other positive effects have been widely evidenced, such as the enhancement of population abundance after stocking (e.g. black sea bream; Blanco Gonzalez *et al.*, 2008a; Blanco Gonzalez *et al.*, 2008b). Harmlessness to wild stocks after releasing hatchery-reared individuals has been reported as well (Jeong *et al.*, 2007; Blanco Gonzalez *et al.*, 2008a; Blanco Gonzalez *et al.*, 2008b; Blanco Gonzalez *et al.*, 2009). In addition, some restocking programs have proved to be economically viable, such as the Japanese flounder (Kitada, 1999a; Okouchi *et al.*, 2004; Kitada & Kishino, 2006), or to be useful for preserving endangered species, such as the beluga sturgeon (Ivanov *et al.*, 1999; Abdolhay, 2004).

1.3.3 Restocking framework

Restocking is one of the options to augment, maintain or restore fisheries production and it should preferably be accompanied by sound fisheries management to achieve a maximum benefit (Born *et al.*, 2004). In biological terms, restocking can (1) increase yield through manipulation of population and/or food web structure, thus raising fisheries production at low external inputs and degree of habitat modification; (2) aid the conservation and rebuilding of depleted or threatened populations; and (3) provide partial mitigation for ecosystem effects of fishing (Lorenzen, 2005). The success in fisheries management is measured with criteria: biological (yield, ecosystem indicators), economic, social, and institutional attributes (Charles, 2001; Garcia & Charles, 2007). Developing successful restocking programs involves far more than producing and releasing hatchery fish that survive. Restocking enters into complex fisheries systems comprising, at a minimum, the target population, the habitat and ecosystem it depends on, the hatchery operation, the fishery (harvesting operation), the markets for inputs and outputs, the stakeholders, and the institutions (rules and regulations) that govern stakeholder behaviour (Pido *et al.*, 1996; Charles, 2001; Lorenzen, 2008).

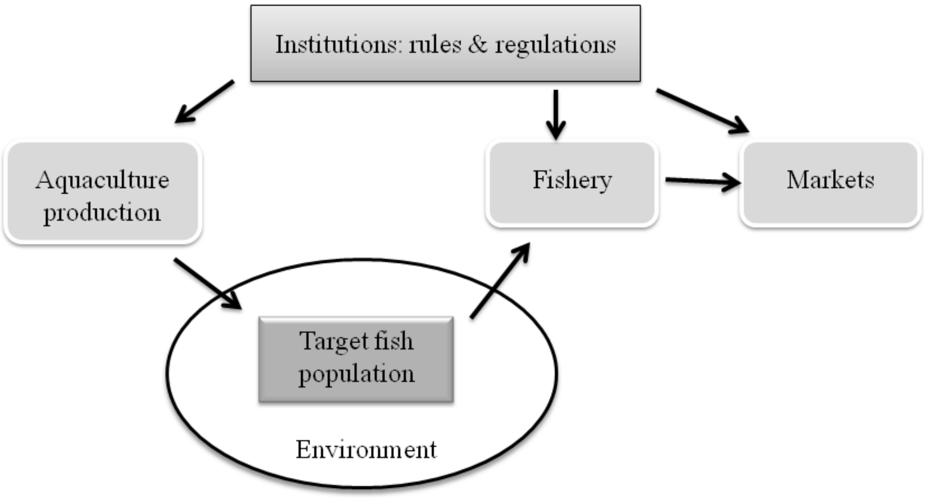


Fig. 1.6.- Framework for analyzing restocking systems.

1.3.4 Restocking strategies

A responsible approach is needed to ensure the success of the restocking program, i.e. a logical and conscientious for applying aquaculture technology to help conserve and expand natural resources. This approach prescribes several key components described by Blankenship and Leber (1995) and shown in the Table 1.3.

Components	Implications
1. Prioritize and select target species	Different driving factors as need by an advocacy group or availability of aquaculture technology.
2. Develop a species management plan	Define the goals and objectives of the program. A feedback loop to evaluate and change production and management objectives.
3. Define quantitative measures of success	Identify success indicators to track progress over time. For example, increase of landings or change in the frequency of rare alleles.
4. Use genetic resource management	Need of increase stock without loss of genetic fitness. This management include: identify de genetic risk and consequences, defining a strategy, implementing genetics controls in hatchery and wild stocks, outlining research needs and objectives, developing a feedback mechanism.
5. Use disease and health management	Important to both the survival of the fish being release and the wild populations of the same species or other species with which they interact.
6. Form objectives and tactics	Consider the ecological factors that can affect the survival, growth, dispersal and reproduction of released fish. For example, predators, food availability, accessibility of critical habitats, competition over food and space, environmental carrying capacity, and abiotic factors.
7. Identify released hatchery fish and assess stocking effects	Use of tags and marks to quantitative assessment the effects of release.
8. Use an empirical process to define optimum release strategies	Pilot release experiments to quantify and control the effect of release variables. For example, fish size at release, release season, release habitat, and release magnitude.
9. Identify economic and policy objectives	Costs and benefits can be estimated and economic models developed. This information can contribute to an understanding with policy makers and the general public about the need of adapted technology and experiments.
10. Use adaptive management	Continued assessment process that allows improvement over time.

Table 1.3.- Components for a restocking program design. Source: Blankenship and Leber, 1995. American Fisheries Society Symposium.

Thanks to advances in fish production and tagging technology, survival can be examined over a range of hatchery practices and release to identify optimum combinations of hatchery and release strategies (Blankenship & Leber, 1995; Munro & Bell, 1997).

1.4 Objectives of the PhD thesis

The pilot release experiments with tagged specimens allow optimizing the effectiveness and assessing the success of the restocking program (Blankenship & Leber, 1995; Svåsand *et al.*, 2000). Therefore, the general objective of this PhD thesis was to evaluate the success of the meagre, *Argyrosomus regius*, restocking program in the Balearic Islands (western Mediterranean). For this purpose, two specific objectives were analyzed in this study. The first specific objective was to develop the methodology needed to produce a sufficient amount of hatchery-reared meagre juveniles for the restocking program (**Section IV**). The second specific objective was to determine the adaptation of the release specimens to the wild conditions, through the study of several parameters such as distribution, feeding, growth, reproduction and survival (**Section V**).

In this **Chapter 1** an overview of the current knowledge about the biology and ecology of the meagre was provided. Also, the restocking as management tool was described.

Chapter 2 describes the methodology used to induce to the spawn hormonally the meagre broodstock and to rear the hatched larvae; in order to obtain every year some thousands of specimens for the restocking program.

Chapter 3 analyses different grow out diets to determine which of them is able to gain grow more effectively and with minimum cost the meagre juvenile until the release size.

Chapter 4 evaluates some physical (T-bar and internal anchor) and chemical (ALR and OTC) tagging methods. The tag retention, the mortality or effect on the fish growth were some of the analyzed factors to identify the released specimens and, so to assess the restocking effectiveness.

Chapter 5 describes the pattern movement observed by the released meagres in the wild, both temporal (day-night pattern) and spatial level.

Chapter 6 analyses, through the study of the condition index, stable isotopes and stomach contents, the time needed for the released meagres to adapt to the wild conditions (i.e., to learn how to capture live preys).

In the **Chapter 7**, the somatic and otolith growth is analysed in the recaptured meagres and compared with the control meagres (kept in captivity conditions), in order to assess whether there are differences in growth into the wild and even if there are differences in survival depending on the growth rate.

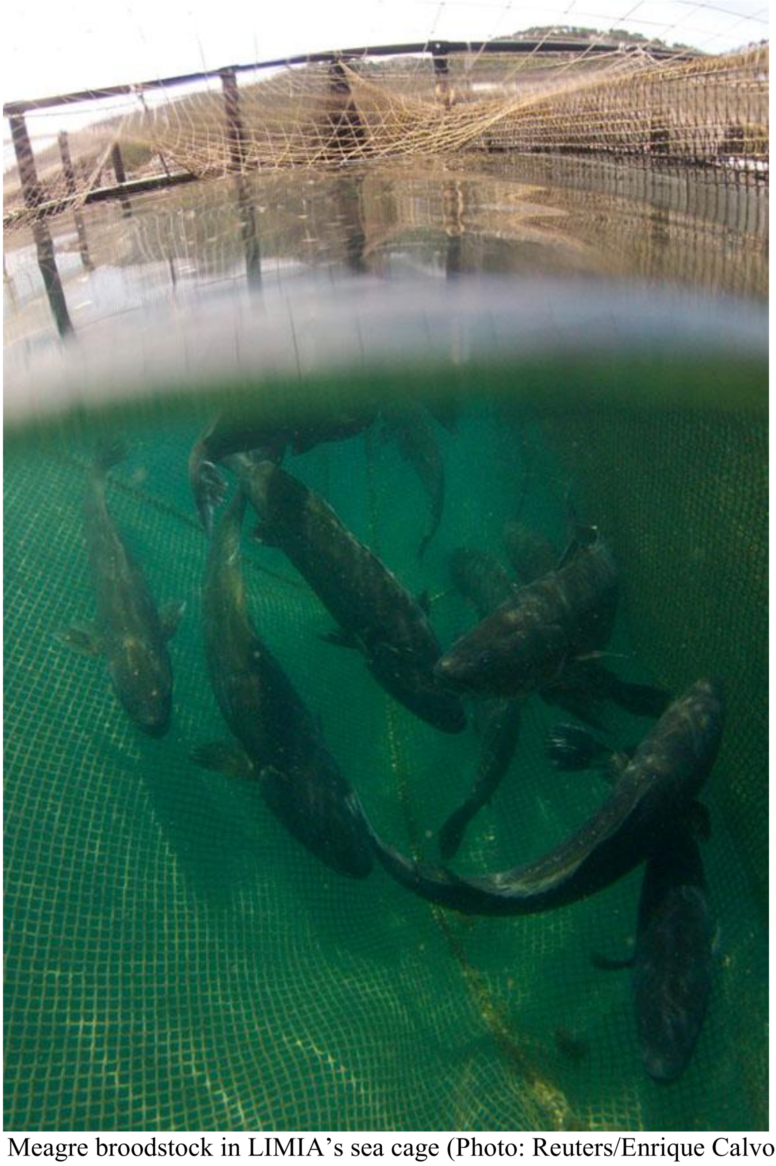
The reproductive parameters are highly plastic and present important spatial differences, therefore, in the **Chapter 8**, parameters as the length at maturity, the spawning season and the fecundity in the Balearic Islands were analysed using reared meagres. Thus, the reproductive behaviour expected for the released meagres was estimated.

Chapter 9 presents estimations of the survival of the released meagres in the Balearic Islands and its implications for the success of the meagre restocking program.

SECTION IV – Methods used for produce, tagging, release and recapture

Production of meagre juveniles in LIMIA facilities (Photo: Jose Mª Valencia)

Chapter 2. Hormonal spawning induction and larval rearing of meagre



Meagre broodstock in LIMIA's sea cage (Photo: Reuters/Enrique Calvo)

In: Pastor, E., Rodríguez-Rúa, A., Grau, A., Jiménez, M.T., Durán, J., Gil, M.M., Cárdenas, S. (Submitted). Hormonal spawning induction and larval rearing of meagre, Argyrosomus regius (Pisces: Sciaenidae). Bolletí de la Societat d'Història Natural de les Balears

Hormonal spawning induction and larval rearing of meagre

Abstract

The aim of the present Chapter was to evaluate the culture potential of meagre, *Argyrosomus regius* (Asso, 1801). Trials were conducted in the LIMIA research centre (Mallorca), beginning in 2000 with the capture of wild meagre juveniles in the Guadalquivir river estuary and growing them to breeders. In May 2006, males with free milt and females with vitellogenic oocytes bigger than 500 µm were injected with salmon gonadotropin releasing hormone analogues (sGnRHa). Spawning occurred approximately 38 hours after induction. Fecundity was high with 1207000 eggs collected from a 13 kg female in a single spawn. Fertilized eggs were incubated at 18.5 ± 1 ºC. The larval development, growth and morphological changes were described from 0 to 30 days post hatching (DPH). Feeding began on 3 DPH, initial swim bladder inflation was observed on 5 DPH, and metamorphosis was completed on 30 DPH. Growth was very fast and the post-larvae reached 15.11 ± 3.49 mm in 30 days. Cannibalism was observed from 15 DPH onwards. These results indicate the success of the meagre production which entails a great importance for the meagre restocking programs.

2.1 Introduction

Sciaenids are generally considered good aquaculture species because they are widely distributed, euryhaline, highly fecund, fast growing and have good food conversion ratios (Silberschneider & Gray, 2008). There has been increasing interest in studying meagre not only as a possible candidate for diversifying commercial aquaculture but also for restocking the depleted natural fishery. The first studies were carried out in France and Italy, and studies began in Spain in 1996 (Calderón *et al.*, 1997). Pastor *et al.* (2002) obtained excellent results growing-on wild (111.8 ± 25.8 g) meagre juveniles in sea cages, which reached 1850 ± 244.9 g in eight months when fed on a diet of fish.

There has only been limited production of meagre fry, with two private hatcheries operating in France and Spain and four experimental public hatcheries operating in

Spain since 2006 (Cárdenas *et al.*, 2008; Pastor & Grau, 2013). The optimal rearing protocol has not yet been determined for this species (Angelini *et al.*, 2002; Quéméner, 2002; Grau *et al.*, 2007; Duncan *et al.*, 2008), however, there are many studies on the reproduction and rearing of other similar sciaenid species, such as mulloway *Argyrosomus japonicus*, shi drum *Umbrina cirrosa* (Mylonas *et al.*, 2000), and red drum *Sciaenops ocellatus*. All of these species exhibit some form of reproductive dysfunction and can only be induced to spawn with hormonal treatments (Thomas & Boyd, 1988; Zohar & Mylonas, 2001).

Controlling the reproductive process is one of the bottlenecks in the development of commercial aquaculture (Zohar and Mylonas 2001). In Spain, attempts to evaluate the possibilities of culturing meagre started in the year 2000 with the capture of wild juveniles in salt marsh ponds of the Guadalquivir River. Here we describe how we controlled the reproduction of the wild caught meagre adults and then reared the larvae. It is crucial to understand these processes in order to improve our knowledge of the biology of this species and to get a large number of meagre juveniles, with optimal cost-effectiveness, for the restocking program.

2.2 Materials and Methods

2.2.1 Capture of breeders

In the autumn of 2000, 94 wild juvenile meagre were caught in the Guadalquivir river estuary (western Andalusia, south coast of Spain). They were held for quarantine and acclimation for a period of six months in tanks supplied with continuous flow-through sea water. During this time they were fed on squid, fresh or frozen fish (*Sardina pilchardus* and *Trachurus trachurus*) and crabs. In April of 2001 the stock was divided into two groups: one with the 50 smallest individuals (111.8 ± 25.8 g mean weight), which was transported in a van equipped with a 0.6 m^3 tank supported with oxygen to the "Laboratori d'Investigacions Marines i Aqüicultura" (LIMIA), Port d'Andratx, (Mallorca, Spain) and stocked in sea cages (Table 2.1); and one group with the remaining 44 specimens, which was held in a tank at the "Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica" (IFAPA), El Toruño, El Puerto de Santa María (Cadiz, Spain).

Holding structures and environment conditions	
Maintenance structures	Cages
Number of structures	1
Volume (m^3)	700
Water depth (m)	5
Min.- max. temp. (ºC)	13-28
Salinity (‰)	37
Breeders	
Origin	Wild
Region of capture	Andalusia
Year	2000
Number of breeders	11
Type of food	Fish
Conservation of food	Fresh / Frozen
Meals per week	4
Initial weight (g)	112
Final weight (g)	11700 – 15000
Stock density ($kg\ m^{-3}$)	0.1
Spawning induction	
Year of induction	2006
Spawning tanks (m^3)	10
Temperature (ºC)	18.5
Salinity (‰)	37
Breeders treated (M/F)	2/1
Mean weight (kg)	10
Hormonal treatment (LHRH)	Injections
Doses of sGnRHa	5 - 10 $\mu g\ kg^{-1}$

Table 2.1.- Conditions in maintaining of the broodstock at the LIMIA centre.

2.2.2 Rearing of breeders

In Port Andratx fish were kept for five years in 700 m^3 sea cages where they were exposed to a natural thermal and photoperiod regime and fed on fresh or frozen fish (*Sardina pilchardus*, *Spicara smaris* and *Trachurus trachurus*). Food rations were given four days per week to apparent satiation.

In February 2003, six individuals (3 kg mean weight) were sacrificed for the histological examination of gonads in order to determine if the fish were maturing in cage captivity conditions. The gonad samples were fixed in 10% buffered formalin, embedded in paraplast, sectioned at 3-4 µm, and stained with haematoxylin-eosin for routine microscopic examination. In December 2005, eleven individuals were selected as breeders, sampled (length and weight) and tagged with AVID microchips. This tagging required capturing the fish with dip nets and transporting them individually in a

0.2 m^3 tank to onshore facilities where they were immersed in an anaesthetic bath of 0.07 g L^{-1} MS222 before processing. All individuals were returned to the sea cages following this procedure after they had recovered from the anaesthetic.

2.2.3 Hormonal induction

The broodstock were sexed in May 2006, which involved examining males for free milt and performing an ovarian biopsy on females with a 2.67 mm diameter plastic nasogastric Levin catheter. The fish were then induced to spawn using injections of Ovaprim© (Syndel Inc., Vancouver, Canada), a commercial product whose active ingredients are the analogue of salmon gonadotropin-releasing hormone [D-Arg^6, Pro^9, Nehylamide]–s GnRHa and a dopamine inhibitor. It was administered intraperitoneally to the abdomen via the rear pelvic fin. One female and 2 male were injected with 10 µg kg^{-1} and 5 µg kg^{-1} of sGnRHa respectively. Both the female and male fish were re-injected after seven and fourteen days, always using the same doses of sGnRHa. During the induction period fish were held in a 10 m^3 indoor rectangular tank, separated of the rest of the broodstock, with continuously flowing sea water (3 m^3 h^{-1}) at salinity 37‰, ambient temperature (18.5 ºC) and fitted with an overflow collector for floating eggs which consisted of 500 µm mesh bags placed below the outflow from the spawning tank. Eggs were collected 6 hours after the spawning to ensure that all the spawn was in the eggs collector.

2.2.4 Larval rearing

Estimations of the number of floating eggs and hatch rates were made volumetrically by taking five 10 mL aliquots of well mixed eggs or larvae from 10 L glass containers and observing them with an Olympus stereomicroscope. The real fertilization success (RFS) was calculated as the total number of spawned eggs (TE) minus the total number of dead eggs (TDE) multiplied by the total spawned $eggs^{-1}$ (TE) multiplied by 100 [RFS = (TE-TDE) TE^{-1} 100], while the apparent fertilization success (AFS) was the number of floating eggs (FE) minus the eggs that died during the incubation period (IDE) multiplied by the number of floating $eggs^{-1}$ [AFS = (FE-IDE) FE^{-1} 100]. Relative fecundity (RF) was estimated as the total spawned eggs (TE) multiplied by the female $weight^{-1}$ (in kg). Hatching success was estimated as the mean apparent hatching rate (AHR) calculated as the number of hatched larvae (HL) multiplied by the number of floating $eggs^{-1}$ multiplied by 100 (AHR = HL FE^{-1} 100).

Floating eggs were separated and disinfected with a 1% iodoform solution prior to transfer to 400 L conical open circulation tanks for incubation. These tanks were maintained at ambient temperature (20 ºC) and 37‰ salinity. Post-hatching yolk larvae were transferred to 1.2 m^3 tanks, and the final larval density was 50 larvae L^{-1}. Tanks were equipped with surface skimmers and surface drainage to clean the surface oil film and ensure swim bladder development. From day 1 to 7, tanks were equipped with a very low continuous 1 µm filtered sea water flow (12 L $hour^{-1}$) that resulted in a 25% day^{-1} water exchange. Then sea water flow was increased progressively until 3 L min^{-1} from day 8 onwards. During larval rearing the temperature ranged between 19 and 23 ºC. At 35 days post hatch (DPH), fish were harvested from the 1200 L tanks and counted. Surviving fish were then placed in 10 m^3 nursery tanks until day 68. The bottom of the tanks was siphoned daily for cleaning from 1 to 68 DPH, and dead fish were counted. After the nursery phase, 68 day-old fish (2.85 ± 1.23 g mean weight) were stocked in a 12 m^3 sea cage for pre-grow out. Survival at 68 DPH was estimated by subtracting the daily dead fish from the number of fingerlings counted at 35 DPH.

From 2 to 16 DPH, larvae were fed with rotifers *Brachionus rotundiformis* cultured with yeast and enriched with DHA protein selco (INVE Aquaculture, Belgium) at a density of 10-20 individuals mL^{-1}, plus green water composed of *Nannochloropsis gaditana* and *Isochrysis galbana* at a density of 80000 - 100000 cells mL^{-1}. Rotifer density was estimated twice a day and adjusted accordingly to maintain the desired concentration. From 10 to 29 DPH, larvae were fed *Artemia* nauplii (grade AF480, INVE aquaculture) at 1-2 nauplii mL^{-1} followed by *Artemia* metanauplii (grade EG, INVE aquaculture) enriched with DHA selco (INVE aquaculture). The quantity of *Artemia* given to larvae was adjusted daily in accordance with their feeding behaviour. From day 23, meagre fry were fed with a commercial weaning diet (INVE NRD 2/4).

Every day up to 17 DPH, a sample of 10 larvae was sacrificed with excess MS-222® for microscopic examination, and from then on every two days up to 30 DPH. The egg diameter, total length (L_T) of larvae, yolk sac length and oil globule diameter were measured with a stereomicroscope provided with an ocular micrometer. Every two days samples were pre-weighed and then dried at 60 ºC for 24 h. Dry weights (W_D) were determined after cooling *in-vacuo* for 1 h. Specific growth rates (% day^{-1}) were calculated using the formula SGR = 100 (ln W_{Df} – ln W_{D0}) t^{-1} (Wootton, 1990), where W_{Df} and W_{D0} are the final and initial dry weights and t the time period in days.

2.3 Results

2.3.1 Induced spawning

Histological analysis of 3 year old meagre gonads showed that 2 of the 4 females (50%) sacrificed in February 2003 had developed ovaries at the final stage of vitellogenesis (Fig. 2.1a) and 100% (n = 2) of the males had free milt (Fig. 2.1b). The remaining 2 females had ovaries in the initial stage of vitellogenesis. Therefore, vitellogenesis appeared to progress in the normal time frame.

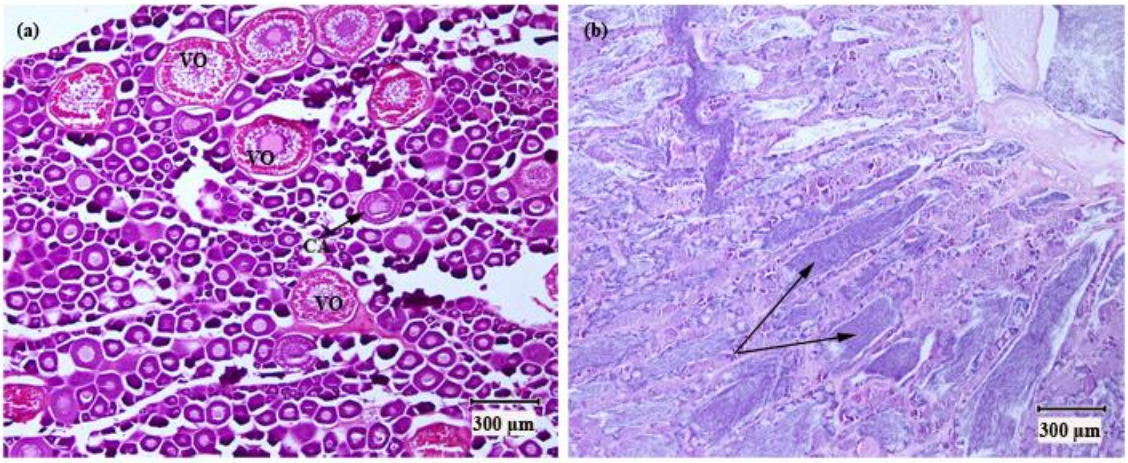


Fig. 2.1.- Transverse section of 3 years old meagre gonads. (a) Ovary in the final stage of vitellogenesis. Note the presence of some vitellogenic oocytes (VO) and cortical alveolus oocytes (CA) between a large population of remaining oocytes at the primary growth stage. (b) Testis with abundant spermatozoa (→) in the spermatogenic tubules.

Of the 11 meagre in the broodstock examined at LIMIA in May 2006, only 1 female had fully vitellogenic oocytes (mean diameter of largest oocytes >500 µm), while 5 males had running milt that could be detected by applying light pressure to the abdomen. It was not possible to determine the sex of the rest of the broodstock. The vitellogenic female (13.0 kg) and two of the running milt males (12.4 kg and 10.0 kg) were injected and kept together in the reproduction tank separated of the rest of the broodstock. Spawning occurred 38 hours after the first injection (HAI). An estimated 1207000 eggs at the blastula stage were collected 6 hours later, with a RFS of 50.7 % (Table 2.2). A second spawn was collected 48 hours later (444500 eggs), and a third one 110 HAI (149000 eggs), although these two last spawns were unviable. The second injection, seven days after the first one, led to the release of 929840 eggs with a RFS of

78.7%. As with the first injection, two more spawns were released 62 (180000 eggs) and 86 HAI (10700 eggs). In this case the average AFR and RFS was not calculated as the eggs were not incubated. With the last injection, 14 days after the first one, fish only released 106000 eggs. The relative fecundity (RF) was 232849.23 eggs kg^{-1} obtained from a single female injected three times in a 15 day period (77616.41 eggs kg^{-1} inj^{-1}). The larval AHR of the two spawns incubated at LIMIA was 69.8% and 72.7%.

Hormonal induction	HAI	Total eggs	% AFS	% RFS	% AHR
First	38	1207000	65.7	50.7	69.8
First	86	445000	0	0	0
First	110	149000	0	0	0
Second	38	929840	84.3	78.7	72.7
Second	62	180000	NV	NV	NV
Second	86	107000	NV	NV	NV
Third	38	106000	NV	NV	NV

Table 2.2.- Meagre spawning results in 2006. HAI: hours after injection. AFS: apparent fertilization success (%). RFS: real fertilization success (%). AHR: apparent hatching rate (%). NV: not valued.

2.3.2 Embryonic development

Fertilized eggs were translucent and buoyant with a mean diameter of 904 ± 49 µm. They had multiple non-pigmented oil globules which coalesced into a single globule at the C-shaped embryo stage (Fig. 2.2a). Eggs were collected early in the morning in the blastula stage, incubated at 20.1 ± 0.4 ºC and hatched 27 hours later.

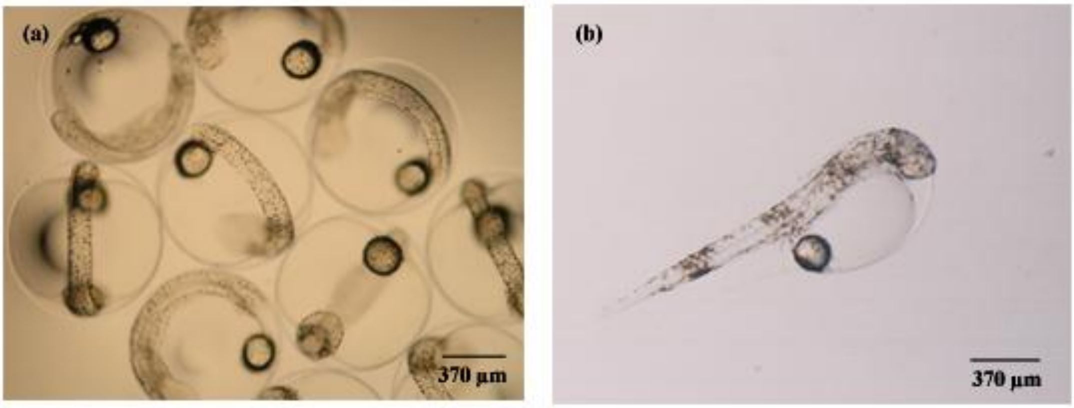


Fig. 2.2.- (a) Eggs in embryonic phase. (b) Recently hatched larva.

2.3.3 Larval development

The length of the newly hatched larvae was 2.22 ± 0.022 mm and they had a mean dry weight of 63 ± 1 µg. The larvae were buoyant, transparent with many chromatophores, and contained a single pigmented oil globule (219.5 ± 0.00 µm) at the caudal end of the yolk sac (1.137 ± 0.057 mm) (Fig. 2.2b). The body was surrounded by the primordial fin and the gut was looped on the ventral side and had no communication with the exterior. After 24 hours the otoliths were clearly visible, the larvae had already consumed half of the yolk sac, and the eyes were easily differentiated. By the second DPH the yolk sac was almost completely reabsorbed, the gut had an opening to the exterior via the anus, and the mouth had started to open, though it still lacked movement. At 3 DPH larvae were 3.3 ± 0.09 mm long, the mouth was mobile and rotifers were observed in the digestive track of some individuals. Pectoral fins started to grow on 4 DPH. On 5 DPH the first larvae with functional swim bladders were observed, and by 13 DPH swim bladders were functional in 91% of larvae. Metamorphosis began by 25 DPH when larvae were approximately 14 mm long, and by 30 DPH 90% of larvae had metamorphosed and were swimming on the bottom of the tank. This transformation marked the end of the larval phase. On 35 DPH fish were harvested from the 1.2 m^3 tanks and counted, with survival estimated at 11.75%. Individuals were stocked in a 10 m^3 fibreglass nursery tank until they reached 2.85 ± 1.23 g mean weight at 68 DPH, after which they were transferred to sea cages for pre-grow out. On 60 DPH the estimated fish survival was 6.58 %.

The following equations represent growth in larval length and dry weight (also see Fig. 2.3a, b):

$$L_{\mathrm{T}} = 2.614\ \mathrm{e}^{0.053\ \mathrm{DPH}} \quad (R^2 = 0.890) \tag{1.1}$$

$$W_{\mathrm{D}} = 0.047\ \mathrm{e}^{0.156\ \mathrm{DPH}} \quad (R^2 = 0.892) \tag{1.2}$$

The mean SGR during larval development was 18.29 ± 6.16% day^{-1}.

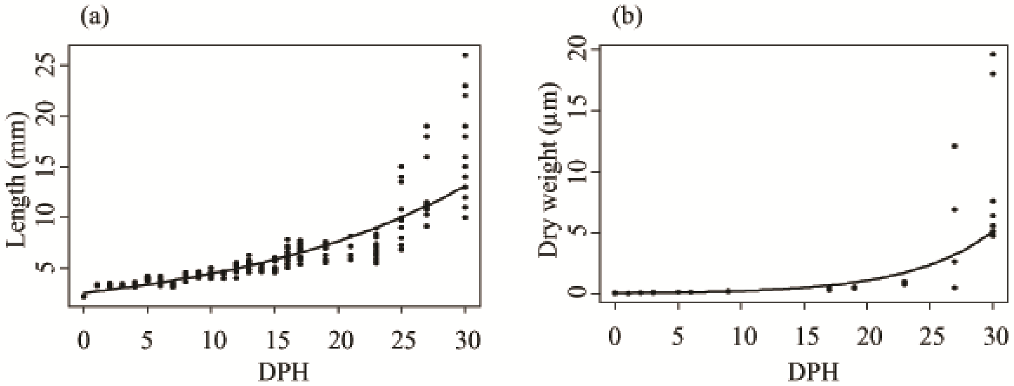


Fig. 2.3.- Growth in larval length (a) and dry weight (b) at LIMIA facilities during the first 30 days post hatch (DPH).

2.4 Discussion

The present study provides data on induced spawning and larval rearing of meagre in captivity under controlled conditions. In the Gironde estuary wild meagre adults aggregate from May to July (Quéro, 1989), and the spawning period generally takes place throughout June and the beginning of July (Quéro & Vayne, 1987) when the water temperature in the estuary changes from 16 ºC to 21 ºC. Meagre is therefore a typical spring spawning species that spawns when both the photoperiod (12-14.5 hours of light day^{-1}) and temperature (16º-21 ºC) are increasing. Spontaneous spawning in meagre was not observed in the LIMIA laboratory. This species therefore does not appear to reach final oocyte maturation in captivity, which is the most common reproductive dysfunction in cultured marine species. Fish exhibiting this type of dysfunction undergo normal vitellogenesis, but with the onset of the spawning season the developing oocytes fail to initiate ovulation and instead undergo atresia (Zohar & Mylonas, 2001).

Different reproductive hormones have been used since the beginning of commercial aquaculture to stimulate reproductive processes and control the spawning of broodstock, including injections of gonadotropic hormones (GTH), pituitary extracts containing GTH, human chorionic GTH and gonadotropin-releasing hormones (GnRH). In some sciaenid species which are unable to breed spontaneously in captivity, treatment with hormones has proven to be an effective method for inducing spawning, such as *S. ocellatus* treated with luteinizing hormone releasing hormone (LHRH) (Thomas & Boyd, 1988; Gardes *et al.*, 2000), *A. hololepidotus* (Battaglene & Talbot, 1994) and *Micropogonias furnieri* (García-Alonso & Vizziano, 2004) injected with human chorionic GTH, and *U. cirrosa* injected with GnRHa (Barbaro *et al.*, 2002). Currently, most commercial fish farmers choose GnRH analogues due to their many advantages. These GnRH peptides are small and use the endocrine pathways of the fish similarly to the native GnRH peptide because they travel from the injection to the pituitary through the blood, and bind to the pituitary receptors with a greater affinity than native peptides (Powell *et al.*, 1998).

Meagre is tolerant of captivity and completes maturation to an advanced stage; it is therefore possible to apply induced spawning protocols successfully (Duncan *et al.*, 2008). In our study meagre was induced with injections of sGnRHa, which led to spontaneous spawning in tanks 38 hours after induction at 18.5 ºC, with eggs released naturally by females for fertilization by males. Duncan *et al.* (2008) did not report data on the time that elapsed from the injection to spawning in his experiences, but comparable results were obtained for the sciaenids, *A. hololepidotus* 32 hours at 25 ºC and 34 hours at 22 ºC (Battaglene & Talbot, 1994), and *U. cirrosa* 34 hours at 24 ºC (Francescon & Barbaro, 1999). Hand stripping was not necessary, which is of great value for aquaculture husbandry as stripping is very time-consuming, laborious and stressful for the fish. Fertilization success and egg viability are also highly variable for batches that are stripped from fish (Norberg *et al.*, 1991; Bromage *et al.*, 1994). Francescon and Barbaro (1999) and Duncan *et al.* (2008) obtained spawning without stripping; however, manual stripping was necessary to obtain eggs and sperm in the induction of *A. hololepidotus* (Battaglene & Talbot, 1994). As we have seen at LIMIA in this study, a mature meagre female can release more than 90000 eggs kg^{-1} in a single spawning event. This high fecundity has also been observed in *A. hololepidotus* (Battaglene & Talbot, 1994), *U. cirrosa* (Francescon & Barbaro, 1999) and by Duncan *et al.* (2008) also for meagre. Photoperiod and temperature are the triggers of gonadal maturation processes in fish. Francescon and Barbaro (1999) working with *U. cirrosa* under experimental conditions described considerable differences in fecundity depending on temperature and photoperiod.

The average diameter of eggs was 904 ± 49 µm at 37‰ salinity, which is consistent with other observations made for meagre (Gamsiz & Neke, 2008) and *U. cirrosa* (Zaiss *et al.*, 2006). Development time is a function of incubation temperature, while egg diameter is correlated with spawning salinity. Many euryhaline sciaenids spawn in estuarine and coastal waters where their eggs are exposed to a wide range of salinities.

For example, spotted weakfish *Cynoscion nebulosus* produce eggs with a small diameter (600 µm) at 45‰ and eggs with a large diameter (860 µm) at 21‰ salinity. A similar relationship was observed for laboratory spawned *S. ocellatus,* which produced eggs with a diameter of 1001 µm at 24‰, 950 µm at 28‰ and 920 µm at 37‰ (Thomas *et al.*, 1995).

The length of newly hatched larvae was similar to observations made by Battaglene and Talbot (1994) for *A. hololepidotus*. Mouth opening was completed at 3DPH, which is slightly earlier than expected for other commonly cultured species, such as gilthead seabream *Sparus aurata* and common dentex *Dentex dentex* in which it occurs at 4 DPH (Elbal *et al.*, 2004; Santamaría *et al.*, 2004), and turbot *Psetta maxima* in which it occurs at 4-5 DPH (Segner *et al.*, 1994). Our results do, however, agree with findings made by Gamsiz and Neke (2008) for meagre and Zaiss *et al.* (2006) for *U. cirrosa*. Presumably, this apparently early mouth opening was due to the faster growth of meagre at the higher rearing temperature. Complete yolk absorption also took place simultaneously with mouth opening.

In terms of the larval rearing stages, meagre appears to fulfil many of the prerequisites for an aquaculture species: the larvae have a large mouth, a very fast growth rate, high survival rates and are easy to handle. The same characteristics have also been described for *U. cirrosa* (Mylonas *et al.*, 2000) and the growth rate was similar to that found for *S. occellatus* (Holt *et al.*, 1981).

In the present study, the ontogenic development of meagre larvae was very similar to that of *A. hololepidotus* and *S. ocellatus* (Holt *et al.*, 1981; Battaglene & Talbot, 1994). This similarity will be helpful for establishing hatchery techniques for meagre because techniques that have already been developed for other sciaenids can be adapted. The feeding schedules used in this study were comparable to those used for *A. hololepidotus* (Battaglene & Talbot, 1994), and larvae reacted very positively to dry food given from day 23, which indicates that they could be weaned earlier.

Early larval mortality was not quantified in this study, but cannibalism was observed from day 15 onwards. Cannibalism is a problem common to most intensively cultured sciaenids (Arnold *et al.*, 1976; Soletchnik *et al.*, 1989; Battaglene & Talbot, 1994) and may be associated with problems of starvation, size dispersion, population density and illumination (Dou *et al.*, 2000; Herrera *et al.*, 2008). In our case, size differences and larval density may have been the main cause of this behaviour.

In conclusion, *Argyrosomus regius* is a very feasible candidate for aquaculture production. The adults of this species have calm behaviour and are therefore easy to handle. They adapt to the conditions of sea cages without problems. Moreover, their reproductive process can be controlled by hormonal induction with sGnRHa, larvae are relatively easy to rear and grow fast. For all these reasons, large quantities of meagre juveniles, needed to carry out a program of meagre reintroduction in the Balearic Islands, can be produced easily and effectively.

Chapter 3. Optimizing production of meagre juveniles

Experimental sea cages at LIMIA facilities

In: Gil, M.M, Palmer, M., Hernández , M.D., Grau, A., Durán, J., García García, B., Jover , M., Pastor, E. (Submitted). Optimizing the production of juvenile meagre (Argyrosomus regius) for a restocking program. Aquaculture.

Optimizing production of meagre juveniles

Abstract

Hatchery-reared juvenile meagre of various lengths are being released in the Balearic Islands (Western Mediterranean) as part of several ongoing pilot experiments conducted under a meagre (*Argyrosomus regius*) restocking program. The production cost of the program depends on not only the size-at-release but also the methodology used to produce the juvenile meagre. For this reason, 7 different diets were tested during the grow-out phase to identify a diet that can produce good-quality juveniles at a relatively low cost. The growth rate, physiological quality (fish condition indices) and nutritional quality (tissue composition) were examined for each diet. Substantial growth differences were observed with the different diets. Diet G (semi-moist diet) provided the best growth, followed by diet A (commercial meagre pellets). However, the small differences observed in the body condition, liver condition and tissue composition of the fish showed that all the diets tested diets, except the experimental diets, produced juvenile meagre of sufficient quality for use in a restocking program. In contrast, the low level of growth, the low condition of the fish and the high amount of mesenteric fat shown on the experimental diets (C, D and E) indicated that these diets were not able to produce quality juveniles. Finally, the reasonable price (unlike diet G) and the superior growth shown on diet A identified this diet as the best option for minimizing production costs at any possible size-at-release.

3.1 Introduction

A large number of species and countries are involved in restocking programs, and this number is steadily growing (Born *et al.*, 2004). The annual budget invested in rearing hatchery fish and releasing them to the wild is not precisely known, but it is in the range of billions of dollars (Brown & Day, 2002). However, restocking has been criticized because it may be neither effective nor economically viable (Bell *et al.*, 2006). Therefore, an appropriate restocking-based management strategy should imply the use of a number of pilot studies to assess the actual effects of restocking on stock enhancement and to develop strategies that maximize the profits of stocking while minimizing its environmental impacts (Blankenship & Leber, 1995).

The initial pilot studies were focused on the development of protocols for breeding adults in captivity and rearing juveniles (Grau *et al.*, 2007; Pastor & Grau, 2013). Moreover, to maximize the survival of the released fishes, various strategies, such as releasing fish at different sizes or with different degrees of biological quality, are also to be examined in subsequent pilot studies (Leber, 1995; Tominaga & Watanabe, 1998; Tsukamoto *et al.*, 1999; Le Vay *et al.*, 2007). Quality not only refers to the release of disease- and deformity-free fish but also implies particular physiological and nutritional characteristics. Physiological welfare can be evaluated by body condition, and biochemical quality is directly assessed by tissue composition (Pepper *et al.*, 1992; Le Vay *et al.*, 2007). Both physiological and nutritional quality have been recognized as factors affecting the capability of hatchery-reared juveniles to adapt to the natural conditions experienced in the post-release environment (Tsukamoto *et al.*, 1999). Release size is also widely recognized as an important factor impacting post-release survival (Leber, 1995; Lorenzen, 2000; Leber *et al.*, 2005). The higher mortality of small juveniles appears to be the result of size-dependent predation rates (Leber, 1995; Johnson *et al.*, 2008). For example, in our case, preliminary results obtained in pilot experiments with *A. regius* have shown a recovery rate close to zero for released juveniles smaller than 20 cm (unpublished data). Although the recovery rate may be dependent on multiple factors (Blankenship & Leber, 1995), it is obvious that size is one of the most important ones (Leber, 1995). However, the size at which fish should be released is also strongly governed by economic constraints. The longer the fish remain in captivity, the greater the cost to feed and house them (Tominaga & Watanabe, 1998; Brown & Day, 2002). Therefore, the optimum release size would result from a trade-off between size-dependent survival rates, growth rates and production costs (Leber, 1995).

Production costs are the most important component of the costs of a restocking project (Ungson *et al.*, 1993). These costs include the maturation, hatchery, nursery and grow-out phases (Leung *et al.*, 1993; Kam *et al.*, 2002). The length of the grow-out phase depends on not only the desired release size but also the growth rate. *A. regius*, like many other species, shows growth rate variations depending on the protein and lipid composition of the diet (Chatzifotis *et al.*, 2010; Estévez *et al.*, 2011). Therefore, the diet selected will ultimately determine the time necessary to achieve the optimum release size and, consequently, the production cost.

This Chapter aims to identify a diet that allows the production of juvenile meagre of good biological quality and of an optimal release size but at the lowest cost. We compared 7 diets, analyzing growth rate, physiological quality (body condition indices) and nutritional quality (tissue composition). Finally, we determined the cost of rearing juveniles over the time needed to reach a given desired size.

3.2 Materials and Methods

Meagre juveniles used in this Chapter were obtained using the protocols of spawning and larval rearing detailed in the Chapter 2. Then, during the nursery and the pre-grow-out phases, first in tanks and later in small sea cages, the fish fry were fed a commercial feed of small grain size (Skretting®, Burgos, Spain; day > 30).

3.2.1 Diet experiments

Juvenile meagre obtained from the pre-grow-out phase were transferred to experimental cages (8 m^3) and given 7 different diets (Table 3.1). However, due to logistic constraints, it was not possible to compare the 7 diets within a single year, and 3 experiments were completed with juveniles born in 2006, 2007 and 2008. Note (see details below) that at least 2 cages per year received the same treatment (diet).

Experiment	Diet tested	Origin
1	A	Meagre commercial pellet (Skretting)
	B	Seabass commercial pellet (Skretting)
2	C	45P/17F experimental composition
	D	47P/20F experimental composition
	E	49P/22F experimental composition
3	A	Meagre commercial pellet (Skretting)
	F	Meagre commercial pellet (Biomar)
	G	Semi-moist diet (OMP, OregonMoist Pellet)

Table 3.1.- Tested diets.

The macronutrient composition of these diets is shown in Table 3.2, where gross energy was calculated using the following energy coefficients (Miglavs & Jobling, 1989): protein 23.6 kJ/g, lipids 38.9 kJ/g and carbohydrates 16.7 kJ/g.

Diet	A	B	C	D	E	F	G
Dry matter	94.34	93.16	91.52	90.79	89.92	92.73	64.87
Crude protein	48.77	49.20	46.12	48.63	49.38	49.17	53.39
Crude fat	18.70	16.62	14.14	17.12	19.51	16.47	20.43
Ash	7.77	6.80	8.98	9.43	9.76	10.44	9.36
Fiber	1.57	1.84	2.10	1.00	0.89	2.10	1.89
NFE[a]	24.13	27.38	28.66	23.82	20.45	21.82	14.93
Gross energy (MJ/kg feed)	22.81	22.65	21.17	22.11	22.66	21.66	23.04
Protein/energy ratio (g protein/MJ)	21.37	21.72	21.78	21.98	21.79	22.70	23.17

Table 3.2.- Proximate composition of the experimental diets expressed as a percentage of dry matter. [a]Nitrogen free extract.

The food ration (*FR*) was continuously adjusted as a function of water temperature, fish size, total biomass and pellet size, following the instructions given by Skretting®. Every month, approximately 20-30 fish per cage were measured (total length, *L*) and weighed (*W*) to monitor the progress of the experiment and to adjust the food ration to the increasing biomass.

3.2.1.1 Experiment 1

During the first year, we compared meagre and seabass commercial pellets, diets A and B, respectively (Table 3.1). This experiment was performed over a period of 11 months (November 2006-October 2007) with juveniles born in 2006. A total of 635 fish (mean *W* ± S.D.: 154.7 ± 48.5 g; mean *L* ± S.D.: 23.7 ± 2.6 cm) were distributed in 4 sea cages (2 replicate cages per treatment).

3.2.1.2 Experiment 2

Juvenile meagre born in 2007 were subjected to 3 experimental diets for 8 months (December 2007-August 2008). These experimental diets were formulated using fish meal, fish oil, wheat and soy oil and produced by extrusion with a Clextral BC-45. The diets presented different protein/fat ratios (45P/17F, 47P/20F and 49P/22F). Approximately 250 fish per cage (mean *W* ± S.D.: 121.3 ± 15.5 g; mean *L* ± S.D.: 21.5 ± 0.9 cm) were transferred to 8 experimental cages. Three of the cages were supplied with experimental diet C, 2 with diet D and 3 with diet E (see Table 3.1 for details on the diets).

3.2.1.3 Experiment 3

The final experiment was performed with fish born in 2008 and lasted for 8 months (February 2009-October 2009). Approximately 87 juveniles per cage (mean W ± S.D.: 95.8 ± 20.9 g; mean L ± S.D.: 20.4 ± 1.4 cm) were stocked in 8 experimental cages. Three cages were fed with experimental diet A (a type of meagre commercial pellet) and 3 with diet F (another type of meagre commercial pellets). Two cages were fed with diet G, a semi-moist diet (OMP, Oregon Moist Pellet) preparation at the LIMIA facilities by mixing raw fish, fish flour and fish oil in a proportion of 10:10:1, respectively. The rations of diet G were adjusted for moisture content such that each treatment received the same ration on a dry weight basis (Millamena, 2002).

3.2.2 Growth

The length-age dataset obtained from the diet experiments was fitted to the growth model proposed by Somers (1988), a version of the conventional Von Bertalanffy growth model that incorporates seasonal growth oscillations (García-Berthou *et al.*, 2012). Additionally, the model was modified to ensure that growth was initiated at the beginning of the grow-out phase. The model used was as follows (note that subindices referencing fish have been omitted):

$$L_t = L0_{year} + (L_\infty - L0_{year})(1 - \exp(-K_{cage}(t - t0_{year}) - S_{t,year} + S_{t0,year}) + \varepsilon_t\,,$$

where

$$S_{t,year} = (CK_{cage} / 2\pi)\sin(2\pi(t - ts_{year}))$$

$$S_{t0,year} = (CK_{cage} / 2\pi)\sin(2\pi(t0_{year} - ts_{year}))$$

$$K_{cage} \sim \text{Normal}\,(K_{year} + K_{diet}, sd_{cage})$$

$$K_{year} \sim \text{Normal}\,(0, sd_{year})$$

$$\varepsilon_t \sim \text{Normal}\,(0, sd_{L_t})$$

and where L_0 is the mean fish length at the beginning of the grow-out phase; L_∞ is the length at asymptotic infinite age; K is the rate of approach to the asymptotic length

(Schnute & Fournier, 1980); t_0 is usually the theoretical age at which the length would be zero, but in our case it is the age at the beginning of the grow-out phase; C modulates the amplitude of the seasonal growth oscillations; and t_S is the time between t_0 and the inflection point of the first sinusoidal growth oscillation.

In our study, L_0 was known (depending on the year, approximately 21 cm or 100 g). L_∞ was assumed to be 171.9 cm, as estimated by González-Quirós *et al.* (2011), although the value of this parameter did not affect the estimates of the parameters of interest, as our tests had previously determined. Depending on the year, t_0 could oscillate between the ages of 6 and 9 months. The remaining parameters (C, K and t_S) were estimated.

These parameters were estimated using a Bayesian approach. A non-informative normal distribution (zero mean and tolerance = 10^{-6}) for K and a uniform distribution for C and t_S were assumed as priors. C was constrained to be within the interval (-1, 1) (García-Berthou *et al.*, 2012), and t_S was constrained to be between 1 and 365 days. Three chains were run using randomly selected initial values for each parameter within a reasonable interval, and conventional convergence criteria were checked. The number of iterations was selected for each run to obtain at least 1000 valid values after convergence and thinning. The models were implemented with the library *R2jags* (http://cran.r-project.org/web/packages/R2jags/R2jags.pdf) of the R-package (at http://www.r-project.org/) that uses the samplers implemented in JAGS (http://mcmc-jags.sourceforge.net/).

3.2.3 Fish quality

At the beginning, in the middle and at the end of the experimental period, a sample of 10 animals/cage was sacrificed following the officially authorized animal care protocol, and data on fish length and total weight, as well as liver weight and mesenteric fat weight, were recorded. These data were used to evaluate the biological condition of the fish on the tested diets based on the relationships between the length and total weight, liver weight and mesenteric fat weight. The sample size for estimating the total weight-length relationship was much larger because the monthly samples obtained to adjust the rations (see above) were also included. The sample sizes are detailed below.

The weight-length relationship was defined by the equation $W = \alpha L^{\beta}$ and was converted into a linear regression by logarithmic transformation. The mesenteric fat was zero in some fish; therefore, logarithmic transformation was possible only by adding a factor (0.01 in our case) to all observations. In all cases, the regression line was forced to pass through the known intercept (W_0 and L_0; note that fish measured at t_0 were only used for estimating mean W_0 and L_0 but not included in the analyses). Therefore, the only parameter of the model was β (the slope):

$$\log(W_i - W_{0_{year}}) = \beta_{cage}(\log(L_i - L_{0_{year}}) + \varepsilon_i$$

$$\beta_{cage} \sim \text{Normal}\,(\beta_{year} + \beta_{diet}, sd_{cage})$$

$$\beta_{year} \sim \text{Normal}\,(0, sd_{year})$$

$$\varepsilon_i \sim \text{Normal}\,(0, sd_W)\,.$$

β_{diet} was determined using a Bayesian approach and the same random-effects hierarchical structure used for K in the previous section. Normal non-informative priors were assumed. Prior to these analyses, the raw data were submitted to an outlier removal procedure, as implemented in the function *influence.measures* of the R package.

The proximate composition of the diet and of the entire fish body were determined with the procedures of the AOAC (1997). After the measurements had been made, all fish samples were frozen at −20 °C until analysis. After homogenization of the individual fish, the crude protein (Kjeldahl method, with a 6.25 nitrogen-to-protein conversion factor), crude fat (ethyl-ether extraction using a SOXTEC System HT6 extractor), moisture (drying at 105 ± 1 °C to constant weight), and total ash (incineration at 450 ± 2 °C to constant weight) contents were determined. The diet ingredients and diets were also tested for crude fiber (Weende method). All analyses were performed in triplicate.

The mean and S.D. values were computed for fish at the end of the 3 experiments. Statistically significant differences between means were evaluated with a one-way analysis of variance (ANOVA) with $P < 0.05$. Post hoc pairwise comparisons were conducted with a Tukey test. These statistical analyses were performed with the R package.

3.2.4 Production costs

To determine the length-dependent economic costs of the meagre grow-out phase for the different experimental diets, the costs related to feeding and personnel were analyzed. Costs (*CO*) were calculated per fish and expressed in euros (€) using the following expression:

$$CO_{diet} = FCO_{diet} + PCO_{diet} .$$

The feeding cost (*FCO*) depends on the accumulated daily food ration (i.e., the amount of feed supplied per day, FR_{day}, Kg, which depends on the temperature, fish size and pellet size) and the price (*P*, €/Kg) of each tested diet. Additionally, because we assumed that the fish were fed on only 74% of all the days, this cost was multiplied by a factor of 0.74.

$$FCO_t = \sum_{day=1}^{t} FR_{day,diet} P_{diet} 0.74 .$$

The prices of the diets were very similar (approximately 1 €/Kg) for all the feeds with the exception of diet G. The raw materials for diet G, especially fresh fish, are more expensive. Accordingly, the cost of the resulting diet is approximately twice that of the other diets. Moreover, due to its higher moisture content, the food ration had to be greater.

The daily food ration (FR_{day}) depends on the fish weight (*W*), which was calculated from the length (*L*) with the model

$$W = \alpha L^{\beta} ,$$

where α and β were determined empirically by pooling several data sets collected at LIMIA.

The personnel cost per fish (*PCO*) depends on the salary per day (*S*), the number of fish housed at a given moment (estimated to be approximately 3000 juveniles per year), the days (*D*) required on each diet to achieve the desired length and the factor 0.74 because the fish were not fed every day.

$$PCO = S * D * (0.74 / 3000) .$$

The costs estimated for each diet allowed us to evaluate the most suitable diet for growing the juvenile meagre to any given desired release size.

3.3 Results

3.3.1 Effect of diets on growth

A total of 5174 length-at-age observations on 3331 fish investigated on 7 different diets were successfully fitted to the proposed model. Initially, this analysis was conducted for each diet and each replicate cage individually to verify that the model provided a good fit to all the cages (Fig. 3.1).

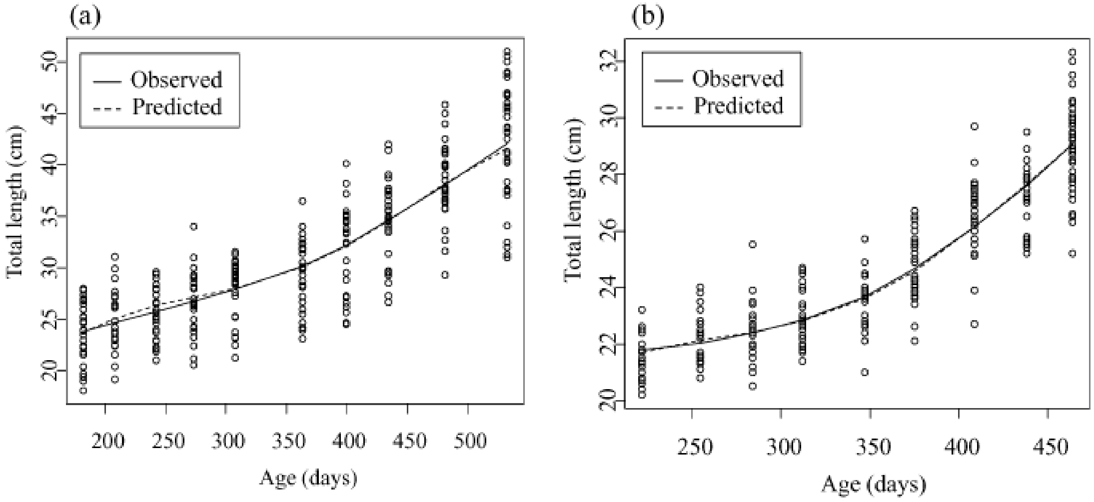


Fig. 3.1.- An example of the fit of the growth model to data from two different cages. (a) Total length of the fish for the first replicate of diet B in experiment 1. (b) Total length of the fish for the third replicate of diet E in experiment 2.

Next, the data were analyzed together as a single data set. The growth results obtained are shown in Fig. 3.2. The best growth was obtained with diet G, although diet A also gave good growth performance. The growth rates of the fish fed the experimental diets (C, D, E) were clearly poorer in comparison with the fish fed the other diets.

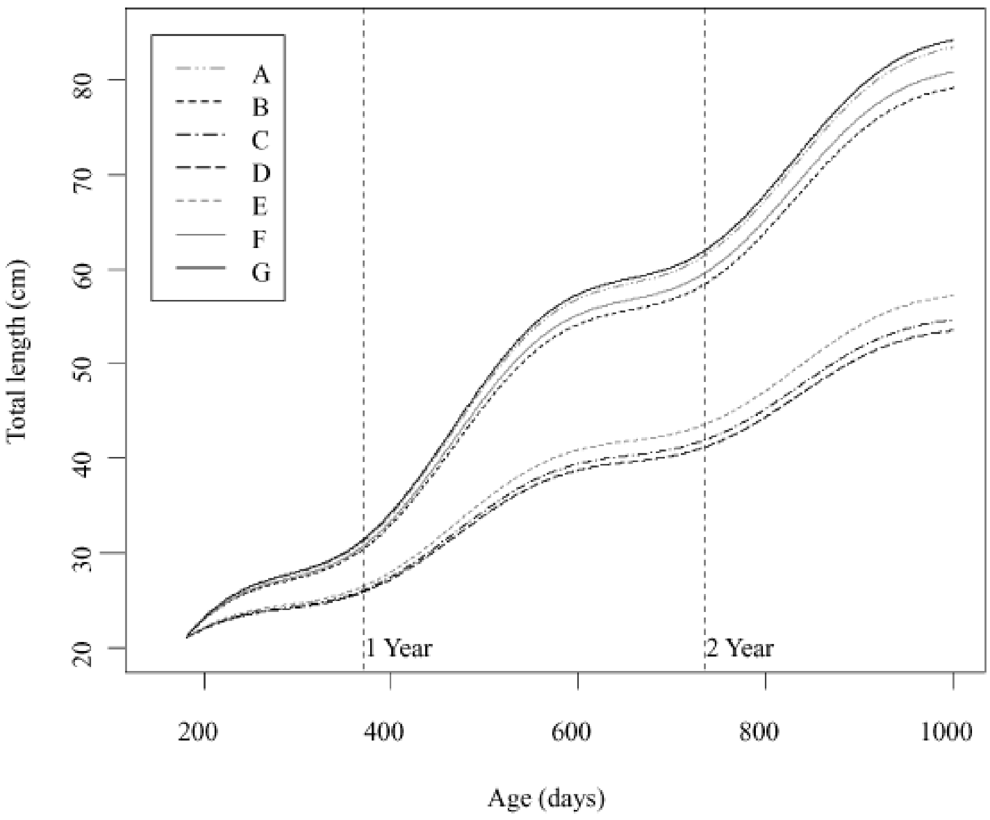


Fig. 3.2.- Growth model of *A. regius* juveniles for the tested diets during the grow-out phase.

3.3.2 Quality of juveniles

The slopes of the weight-length relationships (log-transformed), i.e., total weight, liver weight and mesenteric fat weight, for the different diets and the different years were estimated using the model detailed above based on 5360 fish for total weight and 186 fish for liver and mesenteric fat weight.

The Bayesian credibility intervals of the predicted population average (and their variances) for total weight, liver weight and mesenteric weight for each of the diets and for a desired size-at-release of 30 cm (as an example of plausible desired size) are shown in Table 3.3. These results demonstrated that the value of body condition was best for diet G, followed by diets A, B and F. However, these values differed by only a few grams. For example, the median total weight for a given length of 30 cm was 4.9 g less for diet A than for diet G. Furthermore, the experimental diets (C, D and E) yielded substantially lower weights than the other diets, with differences surpassing 50 g. In the case of the liver weight-length relationship (Table 3.3), diet A presented the highest median liver weight, although the values of liver condition for diets A, B and G were all very close. The results for liver weight were similar to those for total weight: diets C, D and E yielded substantially lower liver weights than the other diets. The mesenteric fat weight was only weakly related to length for any diet, and the pattern of the relationship contrasted with the pattern found for total weight and for liver. For a size-at-release of 30 cm, experimental diets D and E yielded the highest median mesenteric fat weight (Table 3.3). Diet B also provided a considerable quantity of mesenteric fat. However, the other diets yielded median mesenteric fat weights less than 1 g.

	Total weight			Liver weight			Mesenteric fat weight		
Diets	2.5%	Median	97.5%	2.5%	Median	97.5%	2.5%	Median	97.5%
A	289.54	326.64	368.80	5.39	7.65	10.93	0.19	0.80	3.39
B	285.78	323.44	365.61	5.30	7.46	10.54	0.29	1.19	5.08
C	237.03	267.78	302.21	2.61	3.69	5.28	0.17	0.73	3.07
D	241.07	272.33	307.30	3.14	4.45	6.30	0.41	1.69	7.06
E	237.42	267.83	302.69	2.90	4.10	5.82	0.36	1.56	6.43
F	284.81	321.69	363.83	4.77	6.72	9.50	0.08	0.34	1.49
G	293.11	331.58	375.49	5.21	7.44	10.57	0.16	0.67	2.78

Table 3.3.- Summary of the weight-length analysis results for the total, liver and mesenteric fat weight of the different diets for a desired size-at-release of 30 cm, with the median value and lower and upper 2.5% percentiles of the Bayesian credibility intervals for the estimated weights (g).

Significant differences were observed in the ash, moisture, crude fat and crude protein composition of the fish on the 7 diets (ash: $F = 18.8$, $P < 0.05$; moisture: $F = 20.5$, $P < 0.05$; crude fat: $F = 19.4$, $P < 0.05$; crude protein: $F = 11.9$, $P < 0.05$). The crude fat content of the fish on diets A, B, F and G was significantly higher than that of the fish on diets C, D and E (Fig. 3a). Similar results were observed for the crude protein content (Fig. 3b), with average values in a much narrower range (17-18.5%). The crude protein content found on diet F was intermediate in value between the values for the diets with the highest content (A, B and G) and the diets with the lowest content (C, D and E).

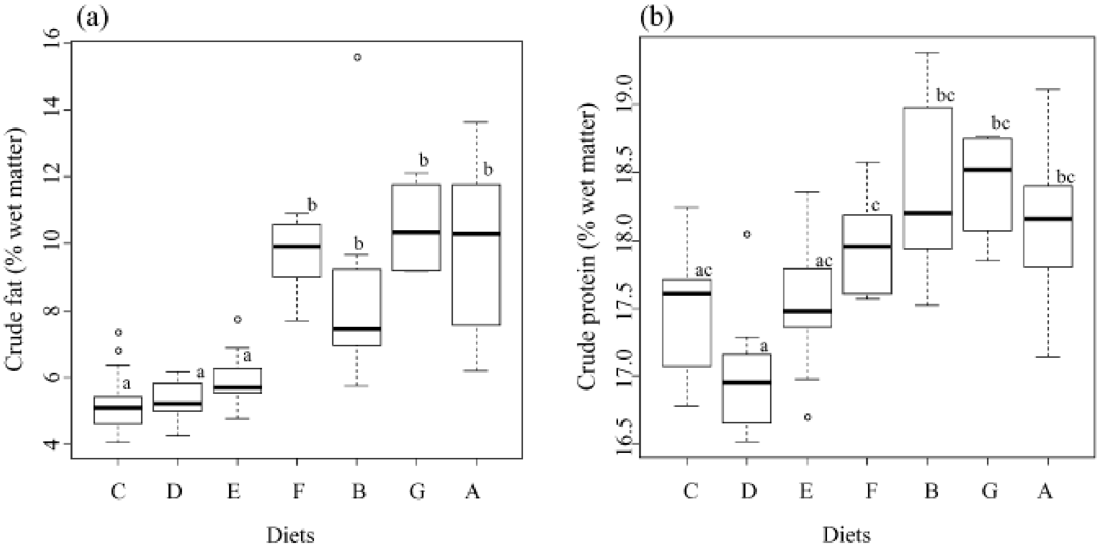


Fig. 3.3.- Boxplots of the (a) crude fat content and (b) crude protein content for the fish reared on the tested diets. A line within the box marks the median values, and the boundary of the box indicates the 25 and 75% percentiles. The results of the pairwise statistical comparisons are represented by different letters.

3.3.3 Production costs

The total costs and the percentage of feeding and personnel costs were obtained for each diet (Table 3.4). As the desired size-at-release increased, the relative proportions of feeding costs and personnel costs varied. However, the proportion of personnel costs was higher than that of feeding costs for all the analyzed lengths. Even in the case of diet G, for which the cost of raw materials was highest, the proportion of personnel costs was greater, although the feeding and personnel costs were almost equal for a length of 40 cm.

	L = 25			*L* = 30			*L* = 35			*L* = 40		
Diets	*FCO*	*PCO*	*CO*	*FCO*	*PCO*	*CO*	*FCO*	*PCO*	*CO*	*FCO*	*PCO*	*CO*
A	9.9	90.1	0.9	13.6	86.4	3.5	17.4	82.6	4.8	18.2	81.8	5.7
B	10.1	89.9	1.0	14.2	85.8	3.7	17.3	82.7	5.0	18.5	81.5	5.9
C	7.9	92.1	2.9	9.9	90.1	5.0	13.0	87.0	6.5	14.0	86.0	9.0
D	8.5	91.5	3.1	10.5	89.5	5.1	13.7	86.3	6.8	15.1	84.9	10.5
E	8.5	91.5	2.7	11.1	88.9	4.9	14.2	85.8	6.4	15.7	84.3	8.1
F	9.5	90.5	0.9	13.2	86.8	3.6	16.4	83.6	4.9	17.3	82.7	5.8
G	26.7	73.3	1.0	34.2	65.8	4.5	41.2	58.8	6.7	42.8	57.2	8.1

Table 3.4.- The total (*CO*, in € fish-1), feeding (*FCO*, in %) and personnel costs (*PCO*, in %) for each diet relative to possible size-at-release values (cm).

Diet A yielded the lowest production cost at any release length (Fig. 3.4). The experimental diets (C, D, E) had the highest total cost because the poor growth on these diets required that the fish be fed for a longer time to reach the desired length than the fish fed with the other diets, implying greater personnel costs. Although the costs of diet G and diet A were similar at the beginning of the grow-out phase, the total cost for producing fish larger than 25 cm with diet G increased substantially. Therefore, despite the better growth furnished by diet G, this diet was not advantageous in terms of the total production costs for any of the possible sizes at release.

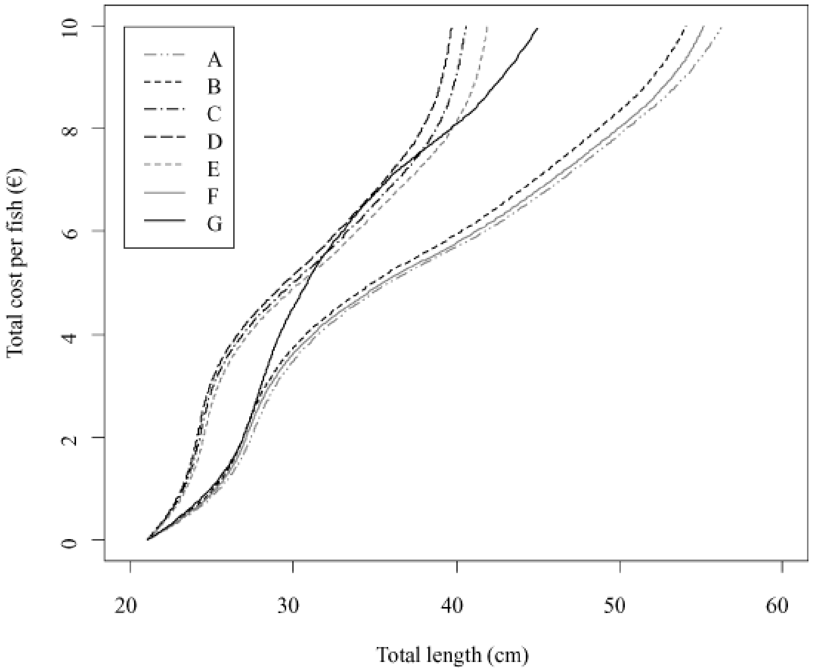


Fig. 3.4.- Size-dependent total cost (€) for a fish fed with the tested diets during the grow-out phase.

3.4 Discussion

Hatchery-reared juvenile meagre are being released at various lengths in the Balearic Islands (Western Mediterranean) as part of several ongoing pilot experiments conducted under a meagre reintroduction program. Size-at-release is one of the most important variables affecting survival and costs associated with stock enhancement (Kellison & Eggleston, 2004; Leber *et al.*, 2005). Once the optimal size-at-release is established, the costs associated with stock enhancement will be minimized when the diet used to raise the juveniles has a low cost, provides good growth and produces good-quality juveniles. Of the 7 diets tested in this study, diet A, although it did not yield the best growth rate, provided the lowest cost for any of the possible sizes-at-release.

To compare the growth rates of juveniles fed different diets was challenging because the fish showed a shift in growth rate following the winter (Fig. 3.1). The growth of most plant species and ectothermic animals, such as fish, reptiles and many invertebrates, is strongly seasonal, depending on temperature, light, and food supply (e.g., Pauly, 1990; Alcoverro *et al.*, 1995; Adolph & Porter, 1996; Coma *et al.*, 2000; Böhlenius *et al.*, 2006). Such the growth of *A. regius* appears to be influenced by seasonal changes. The growth rate decreases markedly at temperatures less than 16 ºC (Quéméner, 2002; El-Shebly *et al.*, 2007). For this reason, the conventional von Bertalanffy growth model did not adequately fit the growth pattern of the juvenile meagre on the diets tested in this study. The observed growth pattern was better described by introducing a seasonal oscillation in preference to the conventional formulation (Somers, 1988; García-Berthou *et al.*, 2012). This method yielded season-independent growth rates that were fully comparable among the diets.

The diet experiments conducted with juvenile *A. regius* revealed that diet G provided the best growth. Diet G, a semi-moist diet, appears to have better palatability than dry pellets. However, the high cost of the semi-moist diet and the required cold storage limit the use of diet G (Kubitza & Lovshin, 1997; Kim & Shin, 2006). The second-best growth rate was obtained with diet A. Additionally, this diet minimized the cost of growing fish to any desired size-at-release.

The expected fitness of the released fish in a natural environment would be the result of both fish quality and a size advantage (Tsukamoto *et al.*, 1999). Although numerous releases of hatchery-reared eggs and larvae were conducted throughout the past century (Svåsand *et al.*, 2000), later experimental releases of genetically marked yolk-sac larvae have shown that the benefits of releasing yolk-sac larva are indeed very small (Kristiansen *et al.*, 1997). In fact, the size-at-release of the fish appeared to have a marked effect on the recapture rates (and presumably the survival) of the released juveniles, due primarily to the greater impact of predation on smaller organisms (Tsukamoto *et al.*, 1989; Ray *et al.*, 1994) but also to the lower resistance to starvation in smaller individuals or to their poorer tolerance of environmental extremes (Sogard, 1997; Hurst & Conover, 1998; Lorenzen, 2000). Therefore, a minimum size requirement is of major importance for the survival of hatchery-released fish (Leber, 1995; Willis *et al.*, 1995). This minimum size, termed the 'critical release size', is defined as the size-at-release below which the probability of survival to reproductive size approaches zero (Leber, 1995). A pilot tag-release-recapture study in the Balearic Islands has shown that cultured *A. regius* with a size-at-release smaller than 20 cm showed a recapture rate close to zero (unpublished data). Therefore, the only fish considered as candidates for release are those larger than 20 cm.

In terms of fish quality, there is a long history of research on hatchery technologies serving to produce high-quality juveniles for not only aquaculture but also stock enhancement (Fushimi, 2001; Shields, 2001). Health status (i.e., disease-free fish), morphology, nutritional status, predator avoidance capability, feeding behavior and genetic makeup are the known factors that determine the ability of hatchery-reared juveniles to adapt to wild conditions (Pepper *et al.*, 1992; Tsukamoto *et al.*, 1999; Fushimi, 2001; Le Vay *et al.*, 2007). Fish quality depends, among other factors, on the composition of the diet. Studies based on red sea bream *Pagrus major* have shown that the quality of hatchery-reared juveniles depends primarily on the nutritional quality of the diet (Nakano, 1996; Le Vay *et al.*, 2007). Accordingly, the current study compared the quality of juvenile meagre fed different diets by studying several variables related to the biological condition of the fish (the relationships between length and total weight, liver weight and mesenteric fat weight) and to the biochemical composition of the tissue.

This study found that the biochemical composition of the tissue (specifically crude fat and protein) was similar and optimal for diets G, A, B and F and low for diets C, D and E. The proximate composition of cultured fish is affected by endogenous and exogenous factors. The levels of protein and ash are primarily related to fish size (endogenous factors), whereas fat depends on exogenous factors, such as diet (Shearer, 1994). The patterns and utilization of lipid storage may reflect the specific life history of the animal (Sheridan, 1994). Although exceptions occur, muscle with a relatively low lipid content is relatively typical of many demersal species. These species are more sedentary than pelagic carnivores, which typically show bursts of muscle output and thus sustain higher lipid levels in muscle tissue to fuel this response (Sheridan, 1988).

Weight-length relationships were used for comparing the 'condition', 'fatness', or 'well-being' (Tesch, 1968) of fish, based on the assumption that heavier fish of a given length are in better condition than thinner fish (Froese, 2006). The fish on diet G showed the best condition, followed by those on diet A. Note, however, that diets G, A, B and F yielded a very similar total weight for a given size-at-release of 30 cm. These total weights differed by only a few grams (Table 3.3). Additionally, note that the within-diet differences were very large in comparison with the among-diet differences. Thus, it is implausible that such small differences would correspond to biologically relevant diet-related differences in the capability of fish to survive at the wild.

In fish, liver is considered a major fat and glycogen deposition site. For this reason, measurements of liver characteristics provide an additional way to estimate nutritional state in fish (Adams & Greeley, 2000). Accordingly, correlations involving liver weight and length are calculated to estimate condition in fish (Benejam *et al.*, 2010). In the current study, the measurements of fish condition based on liver weight were highest and almost equal for diets A, G, B and F, indicating an optimal accumulation of reserves. Chatzifotis *et al.* (2006) have suggested that the liver of brown meagre, *Sciaena umbra*, belonging to the family Sciaenidae, may serve as a depository organ for energy, judging from its relatively high lipid content (39-43%). Alternatively, the lipid content of muscle (1-2.5%) observed in certain species of sciaenids (Poli *et al.*, 2003; Hernández *et al.*, 2009; Grigorakis *et al.*, 2011; Nevigato *et al.*, 2012) indicates the minor role of muscle as an energy storage tissue (Chatzifotis *et al.*, 2006).

The experimental diets (C, D and E) clearly resulted in poorer growth, body condition and liver condition. However, the mesenteric fat weight-length relationships did not show the same pattern. Diets D and E even yielded greater mesenteric fat weight than the other diets, indicating a substantial accumulation of mesenteric fat during the experiment. Piccolo *et al.* (2008) evaluated the effect of two diets with different protein/fat ratios (P/F) and found that, although the biometric traits analyzed were not affected by the diet, the amount of mesenteric fat was significantly higher for the diet characterized by a lower protein/fat ratio. In fish, an appropriate energy-to-protein ratio in the diet contributes to the effective utilization of dietary proteins through the protein sparing effect (Watanabe, 1982). However, meagre does not appear to require high dietary lipid levels (Chatzifotis *et al.*, 2012). At lipid levels greater than 17%, Chatzifotis *et al.* (2010) did not observe a sparing effect on protein. Fish are able to utilize dietary lipids up to a certain level, beyond which growth may be retarded due to reduced feed consumption (Watanabe, 1982). The correlation between dietary lipid and body lipid concentrations has been extensively studied. Too much dietary lipid may result in excessive fat deposition in the visceral cavity and tissues (Lanari *et al.*, 1999). There are indications that meagre has a different lipid metabolism, exhibiting lower fillet fat than the other fish species farmed in the Mediterranean region (Grigorakis *et al.*, 2011). In meagre, as well as in other marine fish species, dietary protein sources may affect the regulation of lipid metabolism (Estévez *et al.*, 2011). In salmonids, increases found in whole-body fat content through the use of dietary plant protein were explained by imbalances in amino acid concentrations (Robaina *et al.*, 1995). In meagre, Chatzifotis *et al.* (2012) found that a dietary increase in crude protein from 40% to 50% positively affected the specific growth rate (SGR) and the feed conversion ratio (FCR). Meagre is a carnivorous species, feeding on Mysidacea, Decapoda and Teleostei in the wild (Cabral & Ohmert, 2001).

In this context, therefore, the mesenteric fat weight-length relationship should not be viewed as an indicator of good condition. In contrast, the other two indices (liver condition and body condition) and the biochemical composition suggest that all the diets evaluated (except the experimental diets) can produce fish of similar quality and apparently fully suited for use in a restocking program.

Economic effectiveness is one of the most frequently criticized aspects of restocking programs, although most restocking programs have yet to be fully assessed because this aspect has received little or no attention (Hilborn, 1998). However, economic viability has been demonstrated in certain cases, e.g., release programs for Pacific salmon *Oncorhynchus* sp. (Isaksson, 1988), Japanese flounder *Paralichthys olivaceus* and red sea bream *Pagrus major* in Japan (Kitada, 1999b; Okouchi *et al.*, 2004; Kitada & Kishino, 2006). From a purely economic perspective, increased returns to the fishery would offset some of the costs associated with improving production and release methods (Brown & Day, 2002). A larger size-at-release is usually preferred because the mortality of smaller fish is greater than that of larger fish. However, the production costs of large fish are high. Furthermore, producing large numbers of large specimens may even be unreliable given the spatial limitations of hatcheries (Kellison & Eggleston, 2004). In contrast, hatchery technology is continuously improving its ability to minimize production costs (Sproul & Tominaga, 1992; Ungson *et al.*, 1993).

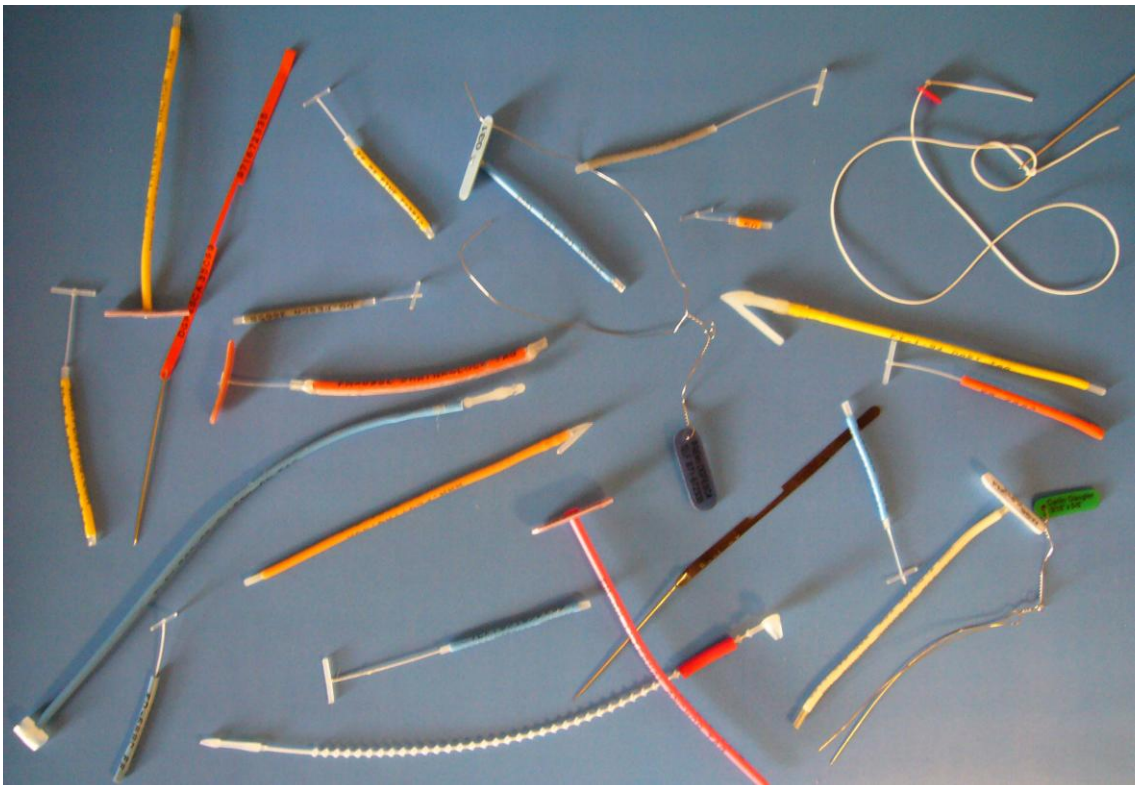
Additionally, improving those aspects of fish quality that may increase survival after release would enable us to reduce the number of fish needed for release, thus decreasing the cost of stock enhancement and minimizing the negative environmental impacts of release (Tsukamoto *et al.*, 1999; Fushimi, 2001).

Diet-dependent survival was not directly estimated in this study because of logistic constraints. However, almost all diets produced fish of similar and good quality (with the exception of the cases cited above). In contrast, the costs of the grow-out phase showed marked among-diet differences. Diet A was the best option for minimizing production costs for any possible size-at-release because this diet has both a reasonable price (unlike diet G) and good growth performance, allowing fish to spend less time in the grow-out phase before attaining the desired size. This last point is decisive because time savings imply reduced personnel costs, which represent the largest component of the total cost (see Table 3.4).

Despite the generally favorable results obtained with diet A, the absolute costs of the grow-out phase were higher than those obtained in other studies of the entire production process (Svåsand *et al.*, 2000; Leber *et al.*, 2005; Patrick *et al.*, 2006). These high costs could be reduced by increasing the number of fish produced or by reducing the size-at-release. However, a substantial decrease in the size-at-release may furnish a stable supply of high-quality juveniles but may compromise survival in the natural environment.

The cost of production is only one of the factors requiring analysis. Other factors that need further study are the magnitude of the release (i.e., the critical number of released fish), the survival of released fish and the economic value of the fish (fisher profits). All of these factors must be considered for a suitable assessment of the cost-effectiveness of a restocking program. Adapting and improving the program in the light of the results of these analyses and incorporating the opinions of different stakeholders may successfully facilitate the design of restocking programs that are socially acceptable, economically viable and environmentally sustainable (Bartley & Bell, 2008).

Chapter 4. Evaluation of different methods for tagging meagre juveniles



Physical external tags

In: Morales-Nin, B., Grau, A., Pérez-Mayol, S., Gil, M.M. (2010). Marking of otoliths, age validation and growth of Argyrosomus regius juveniles (Sciaenidae). Fisheries Research 106, 76-80.

Evaluation of different methods for tagging meagre juveniles

Abstract

The identification of hatchery-reared juveniles is fundamental for assessing the success of releasing events when restocking. An optimal tag should be retained for long time and does not imply any deleterious effect on tagged fish. In this chapter, we compare the effectiveness when tagging meagre juveniles of five different tagging methods; namely, T-bar anchor, internal anchor, visible implant elastomers (VIE), oxytetracycline hydrochloride (OTC) and alizarin red (ALR). Effectiveness was assessed not only in terms of retention time but also in the effect on the fish mortality and fish growth. Internal anchor tags imply high mortality (20.6%). VIE and OTC were ineffective due to low or no retention. ALR is a chemical mark (thus, not detectable by fishermen) but was successful in a mean of 96.8% of the otoliths, susceptible of batch application (bathing), implies low mortality and seems to be permanent. Finally, T-bar anchor tags were lost after some months but they are quickly and easily applicable, has relative low cost and high visibility, the latter being very important to obtain individual information of the recaptured meagres by fishermen. Therefore, a double-tagging method based in T-bar tags and ALR bath was proposed and applied as tagging method for identifying released meagre juveniles.

4.1 Introduction

New improvements for modelling and assessing are becoming widely available and provide powerful and general tools for the evaluation of restocking programs (Lorenzen, 2005; Ye *et al.*, 2005). Nevertheless, one of the most difficult aspects for quantifying the effects on stock enhancement of restocking programs is to identify released individuals. Reared individuals must be efficiently and unambiguously differentiated from wild stocks (Blankenship & Leber, 1995; Chick, 2010). Therefore, the development of reliable tagging methods should precede any investment in restocking itself (Bell *et al.*, 2006).

Moreover, tagging fish not only provides invaluable data for assessing a restocking program but also for other objectives beyond restocking. These objectives range, for example, from determining migration tendencies, studying fish movement patterns or more in general, fish behaviour, validating aging techniques (e.g., interpretation of annual, seasonal or daily growth marks on the otolith), to determining growth rate, mortality rates, and population abundance (Blankenship & Leber, 1995; Munro & Bell, 1997; Bell *et al.*, 2005; Phelps & Rodriguez, 2011). Therefore, a relevant background on fish tagging methods have been developed, and on the basis of this background a number of key recommendations have been distilled when choosing a tagging method: (1) tags should be easy to apply and identify, (2) should allow to identify individual fish or, at least, cohorts or groups of fish, (3) should be retained by fish through time, (4) should be cost-effective, and (5) should not significantly affect neither fish mortality, growth nor behaviour (McFarlane *et al.*, 1990; Catalano *et al.*, 2001). Unfortunately, none of current tagging methods is universal in the sense that it guaranties these features for any fish species. By contrasts, some of these features are highly species-specific and, therefore, the most appropriate tagging method should be specifically analyzed for each species.

Tagging methods can be clustered within 4 broad categories: electronic, biological, physical, and chemical tags (Bell *et al.*, 2005). (1) Electronic tags can be as simple as implanting a passive integrated transponder (PIT) tag, to the use pop-up satellite archival tags. Archival data storage tags (datalogger) can record temperature, depth and salinity, and acoustic tags have been miniaturized and receivers can be spaced across more or less large areas in order to recover fish position at different spatial scales (Leber & Blankenship, 2011). However, electronic tags are generally expensive methods which allow marking a few fish, although they are widely used in studies of movement patterns (Chapter 5; March *et al.*, 2010; Alós *et al.*, 2011; March *et al.*, 2011; Alós *et al.*, 2012; Cabanellas-Reboredo *et al.*, 2012). (2) Biological tags can range from heritable genetic markers, presence of areas-specific parasites to external morphological marks. Biological marks may be either natural or imposed from diet, branding, or removal of some physical structure from an individual (e.g., tail or fin clipping). Microsatellite DNA has been proved to be useful for batch marking all life stages, from eggs through adults (Leber & Blankenship, 2011). Rapid advances are being made in genetic marking systems and these systems have enabled even tracking fish across generations (Ward, 2006; Leber & Blankenship, 2011). (3) Physical tags refer to identifying labels that are fixed to or inserted through external structures (Chick, 2010) and are externally visible. High external visibility is desirable when commercial or recreational fishers are expected to return tagged fish. Physical tags can be divided into external and internal tags. External tags allow a rapid individual or group identification and have the advantage of being easy to apply, economical and its application does not usually require sophisticated technology (Astorga *et al.*, 2005). Barbed dart tags or T-bar anchor have been extensively used (Phelps & Rodriguez, 2011). Internal tags have been also used with a wide range of fish and they appear to have a higher retention rate than the external tags (Catalano *et al.*, 2001; Astorga *et al.*, 2005). The visible implant elastomers (VIE) and the visible implants Alpha (VI Alpha) tags are the most commonly used internal tags. (4) Chemical tagging can range from stable isotope analysis or microchemical analysis to immersion in or injection of fluorescent compounds. Batch marking by immersion in fluorescent compounds bind to the growing edge of hard structures, as the otoliths, and allows the marking of large numbers of specimens with low cost. Otolith marks have been produced in juvenile fishes using chemicals such as oxytetracycline hydrochloride (OTC; McFarlane & Beamish, 1987), alizarin complexone (ALC; Reichert *et al.*, 2000), alizarine red (ALR; Simon *et al.*, 2009) and calcein blue (Bumguardner & King, 1996), among others.

Ideally, tagging should not influence survival, growth or behavior of the fish, nor should tag loss occur. However, tag-loss occurs frequently and averaged retention time typically depends on the type of mark. Tag loss may cause an underestimation of the survival rate for the hatchery-reared juveniles and, therefore, an underestimation of restocking success. Tag-loss rate can be estimated by releasing double-tagged fish (Wetherall, 1982; Otterå *et al.*, 1998). The advantage of these methods is that they only use ratios of numbers of individuals recaptured with one of the two marks, or both, thus they are robust to migration, mortality and failed detection, provided these processes have the same rates for all tagged fish (Wetherall, 1982; Venerus *et al.*, 2013). Some of these double-marking techniques require the use of permanent tags, as VIE (Hartman & Janney, 2006) or coded wire tags (CWT; Henderson-Arzapalo *et al.*, 1999), in order to distinguish which individuals have lost the tag from those that were never tagged.

In this Chapter we evaluated which are the most appropriate tagging methods for meagre juveniles. The rates of tag loss, survival or growth were investigated for some physical tags (T-bar anchor tags, internal anchor tags and VIE) and some chemical ones (OTC and ALR). The efficiency of the physical tags was analyzed through the information of the recaptured specimens given by the commercial and recreational fishers, and by monitoring some tank experiments. Instead, the efficiency of the chemical tags was evaluated by observing the tag presence in the otoliths of specimens subjected to experimental baths. In addition, other aspects such as material and personnel (i.e., time) costs of application were taken into account in order to select the most effective tagging method for meagre juveniles.

4.2 Material and Methods

4.2.1 Physical tags

4.2.1.1 Tags efficiency in the wild

The T-bar anchor tags are commonly used in many restocking studies (Bianchini *et al.*, 2001; Støttrup *et al.*, 2002) because they can be applied quickly and easily (Scheirer & Coble, 1991). Meagre juveniles were tagged with Floy® T-Bar Anchor FF-94 in the case of fish less than one year old and FD-94 tag for the rest (Fig. 4.1a). Each external tag was imprinted with an identification number and phone number to call when the fish was recaptured. These tags were inserted with a tagging gun under the dorsal fin on the left side of the fish. The needle penetrated the fish to a depth great enough to allow the "T" bar to become secured behind one or more of the interneural bones (Fig. 4.1b). Fish anesthesia was not necessary for this tagging method.

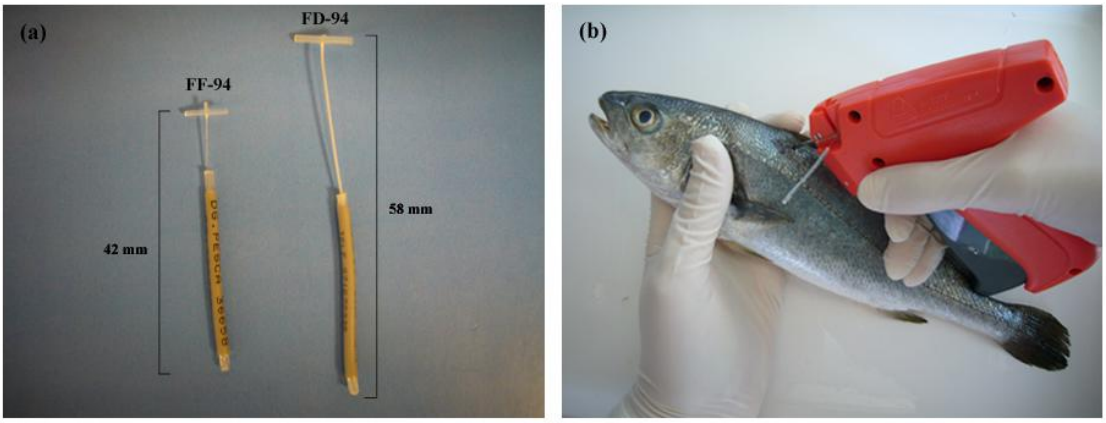


Fig. 4.1.- (a) T-bar anchor tags used in meagre restocking. (b) Procedure for tagging meagre with FF-94 T-bar anchor tag.

4.2.1.2 Tags efficiency in tank experiments

Three tank experiments were conducted using tagged meagre juveniles subjected to different conditions such as fish length, diet, type of external tag or temperature.

The first experiment started on October 2009 when the juveniles were 17 months old (age-1+), with a mean total weight (W_T) of 570.6 ± 69.1 g and with a mean total length (L_T) of 37.3 ± 1.4 cm. In this experiment, 20 juveniles were stocked during seven months in a 5000 L tank divided into two sections. The tank circulation was open, and the water temperature was maintained at 19.7 ± 1.3 ºC with the help of a heating system. The fish were tagged with T-bar anchor tags and distributed in two replicates (10 meagres per group). Fish were fed daily with fresh food (until satiation). All fish were sampled twice a month. At each sampling date, W_T and L_T were measured and the retention of the T-bar tag was checked.

The second experiment began on October 2010 and was conducted with 5 month old juveniles (W_T = 83.7 ± 24.5 g and L_T = 19.5 ± 1.9 cm). A total of 60 juveniles were tagged with T-bar anchor tags and distributed in a 10000 L tank divided in 6 sections (10 meagres per group). The tank circulation was open, and the water temperature was maintained at 18.0 ± 1.3 ºC with the help of a heating system. The fish were subjected during almost 4 months (112 days) to three different treatments: control, starvation and low diet; with two replicates for each treatment. Control fish were fed daily with fresh food (until satiation) and the starved fish were not fed throughout the experiment. Poor diet fish were fed once a week with fresh food. Once a week, all fish were measured (W_T and L_T) and the retention of the T-bar tags was checked.

The third experiment was a double-tagging experiment. This method is based on the use of permanent tags, as passive integrated transponders (PIT) or visible implant elastomers (VIE) (Josephson *et al.*, 2008; Rude *et al.*, 2011), because they allow to identify the fish that have lost the external tag. The tank experiment was carried out during 139 days with 8 month old juveniles (W_T = 147.7 ± 31.8 g and L_T = 24.4 ± 1.6 cm). The tank circulation was open, and the water temperature was not controlled (16.0 ± 2.0 ºC). A total of 90 juveniles were held in a 10000 L tank divided in three sections (30 meagres per section). Each section contained 10 fish for each of the three tested treatment: (1) control fish, (2) fish tagged with orange VIE and T-bar anchor tag, and (3) fish tagged with green VIE and internal anchor tag. Control fish were measured but not anesthetized. The rest of fish were anesthetized (0.5 mL L^{-1} 2-phenoxyethanol) and measured just prior to the tagging. The internal anchor tag (Model FM-95W; Floy Tag Company, Seattle, WA) consisted of a plastic 4.7 x 19.2 mm elliptical disk and a 43.2-mm imprinted streamer (with identification number and phone number). To apply the internal anchor tags, a scalpel was used to make an approximately 6-mm vertical incision into the abdominal wall, inserting the tag into the body cavity, and allowing the incision to close naturally (no suture stitches were applied; Fig. 4.2a). The visible implant elastomer (VIE; NWMT; Shaw Island, Washington) is injected as a fluorescent liquid polymer into transparent tissues of the study organism where it solidifies after injection (Bailey *et al.*, 1998). After experimenting with various tagging locations in meagre, we chose to insert manually VIE marks into the left inferior opercular area (Fig. 4.2b) where the skin is thinner and whiter. The T-bar anchor tags were inserted as described in Section 4.2.1.1. All fish were sampled twice a month, where W_T and L_T data were taken, and the retention of the tags and healing of the internal anchor tags were checked.

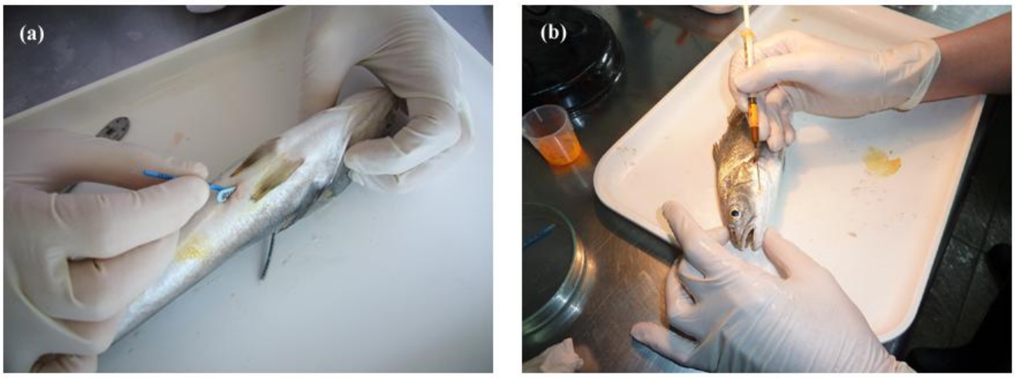


Fig. 4.2.- (a) Internal anchor tag (FM-95W) application in the abdominal cavity. (b) Injection of orange VIE in the opercular area of meagre juvenile.

4.2.1.3 Data analysis

4.2.1.3.1 Retention tag

Retention time of fish released in the wild can be estimated using the information from the recaptured meagres because it is assumed that all recaptured meagres in the Balearics come from the restocking program. Information campaigns aimed at the commercial and recreational fishermen, as well as the monitoring of meagre catches in the fish market, allowed maximizing the information obtained about tagged and untagged recaptured meagres. With the identification number in the case of tagged recaptures and with the ALC mark at the otolith in the case of untagged recaptures, it was possible to know the time-at-liberty spent by the recaptured fish. However, reporting rate of tagged and untagged fish may differ, thus these data may be biased.

The three tank experiments provided unbiased data of T-bar retention in meagre juveniles. The different conditions of each experiment allowed establishing the influence of different factors in the retention time (Table 4.1).

Factor	Experiment	Treatment
Fish length	1	Control
	2	Control
Diet	2	Control
	2	Starvation
	2	Poor diet
Temperature	2	Control
	3	T-bar

Table 4.1.- Factors tested to evaluate their influence in the T-bar anchor tag retention and samples used for each factor.

The fish length was analyzed comparing age-0+ and age-1+ fish. Diet effects were analyzed by comparing the fish submitted to different amounts of food, and the temperature was analyzed by comparing fish subjected to a controlled temperature around 18 ºC and the fish subjected to the natural temperature cycle (16 ºC on average).

The differences in the T-bar tag retention time between treatments for each of these three factors were analyzed using survival analysis method. Survival analysis examines and models the time it takes for events to occur (Crawley, 2007). Originally, the method was developed for medical survival analyses but has already been applied in other fields. In order to determine differences in the tag retention rate between treatments, a proportional hazards model (or Cox regression model) was applied to the data. This model describes the probability per time unit of the event occurring as a function of a basic probability (the baseline hazard) and a set of explanatory variables (e.g. fish length, diet or temperature). The model is non-parametric, and it is not necessary to make assumptions about the distribution of the 'time to event'. The probabilistic nature of the model allowed predicting the variation in the timing of the event (here, the percentage of tag retention). The *coxph* function from the survival library of the R package (developed by T. Therneau and T. Lumleyat; http://cran.r-project.org/web/packages/survival/survival) was used to estimate the model parameters and likelihood ratios. Predicted survival rates at different times and treatments were estimated using the function *survfit* from the same library.

4.2.1.3.2 Growth effect of tags

The fish length dataset taken every 15 days during the third experiment explained above (Section 4.2.1.2) was used to compare the effect of the T-bar and the internal anchor tags on the fish growth, using the untagged fish as a control.

A version of the Von Bertalanffy growth model allowing for seasonal oscillations and fully described in Chapter 3 was applied to this data. In this case, L_0 was obtained from total length of the fish at the beginning of the experiment (24.5 cm) and t_0 was the fish age at the beginning of the experiment (263 days). As in the Chapter 3, L_∞ was assumed to be 171.9 cm (González-Quirós *et al.*, 2011) and the other parameters (C, K and t_S) were estimated using a Bayesian approach. The values of the parameters C, K and t_S obtained for meagres fed with the used diet (A; commercial meagre pellets; Chapter 3), were assumed as priors. As in Chapter 3 the model was also implemented with the library *R2jags* of the R-package.

Growth differences were analyzed comparing the K values obtained for the fish tagged with T-bar, the fish tagged with internal anchor tag and the control fish. The K parameter is a measure of the exponential rate of approach to the asymptotic length (Schnute & Fournier, 1980); therefore, it is not exactly a the growth rate.

4.2.1.3.3 Mortality of tags

All the tagged fish for the restocking program remained 5-7 days in experimental cages previous to release to detect short-term mortality produced by the tagging process. Therefore, the number of tagged fish and the number of dead fish was known for each release event (n = 21). So, the probability of dying in the i release event (p_i) for the T-bar tags was estimated. The number of observed dead fish ($n.died_i$) in a release event was assumed to be binomially distributed as:

$$n.died_i \sim \text{dBinomial}(p_\text{i}, n_i)$$

The (logit) transformed p_i was assumed to be normally distributed around an averaged (across events) *mean.p*:

$$p_{\mathrm{i}} = e^{\mathrm{logit.p_i}} \Big/ (1 + e^{\mathrm{logit.p_i}})$$

$$Logit(p_{\mathrm{i}}) \sim \mathrm{dNormal}(Logit(mean.p_{\mathrm{i}}), sd_i)$$

where *mean.p* and its standard deviation were estimated using a Bayesian approach. Non informative normal distribution (zero mean and tolerance = 10^{-6}) for *mean.p* and uniform distribution for *sd* were assumed as priors. The model was implemented with the library *R2jags* of the R-package.

Only one of the release events (Release 20, see Appendix Table I.1) was carried out with meagre juveniles tagged with internal anchor tags. The probability that the observed mortality for the internal anchor tag was the same estimated for the T-bar tags was tested using a Monte Carlo simulation. For this, 1000 simulations for the 363 fish tagged with internal tag were generated using the parameters estimated for the T-bar mortality distribution.

4.2.2. Chemical tags

Morales-Nin, B., Grau, A., Pérez-Mayol, S., Gil, M.M. (2010). Marking of otoliths, age validation and growth of Argyrosomus regius juveniles (Sciaenidae). Fisheries Research 106, 76-80.

4.3 Results

4.3.1 Physical tags

4.3.1.1 Retention rate in the wild

Information of 356 recaptured meagre was used to estimate the T-bar tag retention in the wild. 329 of the reported recaptures still retained the T-bar tag. However, 27 of them had lost the tag. Although, via the length or weight information given by the fishermen or via the otolith observation of the recaptures returned to the laboratory, the release date could be unambiguously established for 19 untagged fish. The remaining 8 untagged fish were not included in this study.

Retention rate of T-bar anchor tag was near of 100% until 180 days-at-liberty, although some tag losses were detected few days after release. The percentage of tag retention markedly decreased in the fish that spent more than 180 days-at-liberty (Fig. 4.4). Despite the observed results, the returned rate of the tagged and the untagged meagres can not be considered equal; therefore the T-tag retention rate could be less than that observed because untagged fish may have more chance to remain unreported than tagged fish.

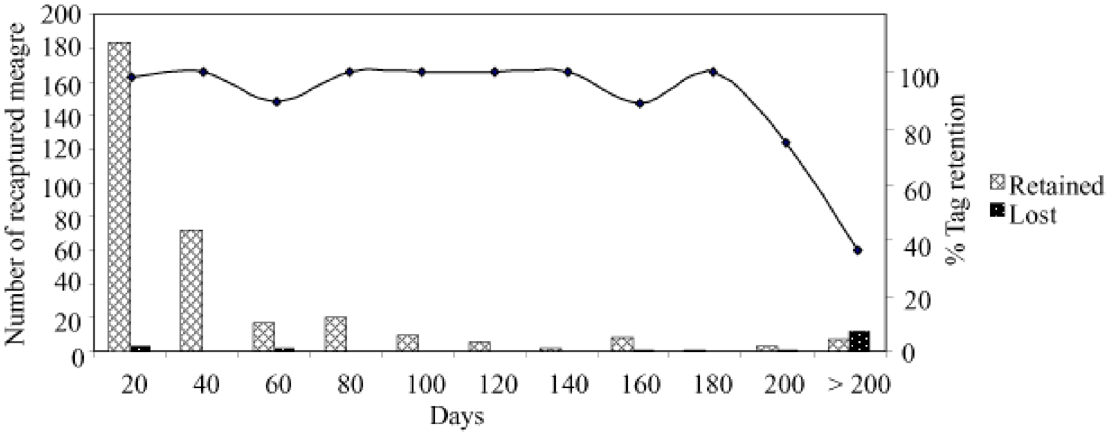


Fig. 4.4.- Evolution of the number of meagre recaptured in the wild with retained tag and with lost tag, and the percentage of tag retention.

4.3.1.2 Retention rate in tank experiments

The tag retention rate fitted using the Cox model was estimated separately for each factor (i.e., fish length, diet and temperature). Probabilities of tag retention over time were found to be significantly affected by all these factors (Table 4.2).

Factor	n	Coefficient	d.f.	P
Fish length	40	17.66	1	< 0.05
Diet	60	20.48	2	< 0.05
Temperature	50	17.7	1	< 0.05

Table 4.2.- Cox regression coefficients for the three factors compared with the tank experiment fish.

The tag retention for the larger fish (age-1+) was significantly higher than the smaller fish (age-0+), which have a 0% of tag retention around 200 after tagging date (Fig. 4.5a). The diet (i.e., amount of food given to the fish) influenced significantly the tag retention. Fish subjected to a poor diet presented higher tag retention than the control fish fed daily, and the starved fish retained almost all the tags (Fig. 4.5b). Finally, the temperature appears to have a significant effect on the tag retention because the fish subjected to low temperatures (natural) presented higher tag retention than the fish with controlled temperature (Fig. 4.5c).

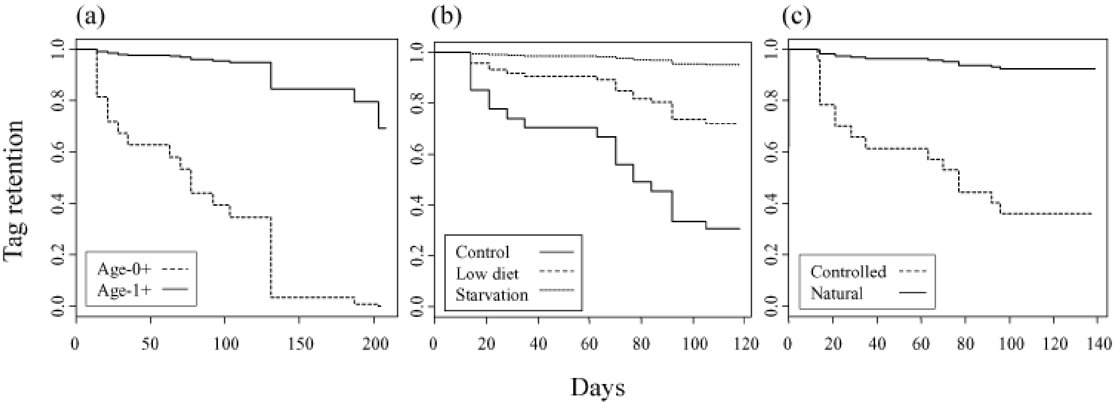


Fig. 4.5.- Estimated tag retention for the three tested factors. (a) Fish length; comparison between the control fish of the two size-groups (age-0+ and age-1+). (b) Diet; comparison between the three tested diet conditions: control, low diet and starved. (c) Temperature; comparison between fish subjected to a higher and controlled temperature and fish subjected to a lower and natural temperature.

The fish tagged with internal anchor tag in the third experiment were not included in the analysis because no tag was lost during the course of the experiment (139 days). Instead, the VIE, which was expected to be permanent during all the experiment, presented a high tag loss (54.1%) at the end of the experiment (139 days).

4.3.1.3 Growth effects of tags

The Bayesian inference adopted here facilitates the estimation of individual growth parameters and between-fish variability for the fish tagged with T-bar and internal anchor tag, and control fish. The *K* parameter (i.e., the exponential rate of approach to the asymptotic length) was similar for all the treatments (Fig. 4.6), indicating that there are not differences in the growth of fish tagged with different types of tag.

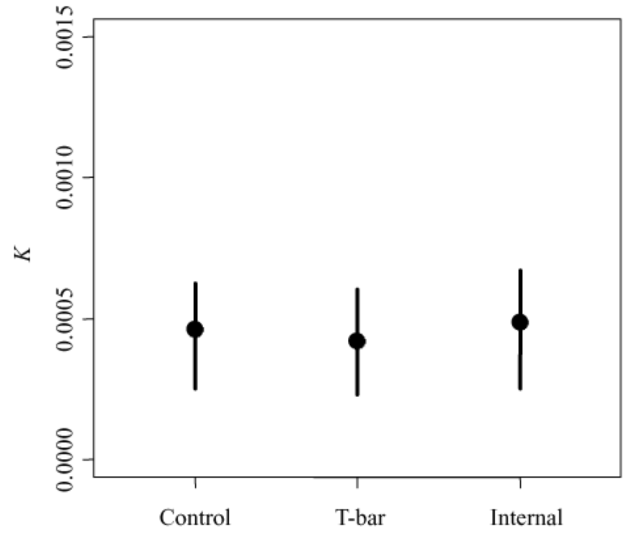


Fig. 4.6.- Values of the parameter *K* estimated for the von Bertalanffy growth model with seasonal oscillations for the control fish and the fish tagged with T-bar and internal anchor tags in the tank experiment. The dots are the median values and the vertical lines the 2.5 and 97.5% percentiles.

4.3.1.4 Mortality of the tagging process

Concerning the fish to be released, different mortality rates, ranging from 0 to 9.9%, were observed in the different releasing events. Remember that this mortality refers to short term, that is, the meagres were tagged and remained in an observation tank for a few days just before they were released into the wild. The estimated mean mortality of all the release events (excepting the case of Release 20; see below), had a mean of 0.016 (1.6% of fish died), with an standard deviation (at the logit scale) of 1.5.

These parameters were used to test if T-bar related mortality is different to the (unique) estimate of mortality related with implanting an internal anchor tag. The existence of differences was evaluated via Monte Carlo simulations: 1000 values of random trials of consisting in simulating how many of 363 specimens (those tagged with an internal anchor tag) are expected to die if they were marked with T-bar tags. The distribution of the 1000 values of expected number of dead fish were compared with the currently observed value (75 from 363 or 20.6%) showing that the probability of obtaining the observed value by change is very small ($P < 0.05$), thus demonstrating that the application of internal tags on meagre juveniles produced a higher mortality than the application of T-bar tags.

4.3.2. Chemical tags

Morales-Nin, B., Grau, A., Pérez-Mayol, S., Gil, M.M. (2010). Marking of otoliths, age validation and growth of Argyrosomus regius juveniles (Sciaenidae). Fisheries Research 106, 76-80.

4.4 Discussion

A distinct and persistent tag enables released fish to be identified by fishermen, which increases the chance that released fish could be returned. High return rate is fundamental for any demographic study in which individuals are released and recaptured. Specifically, returned fish are needed for enhancing the success of a restocking program because the conventional tools for assessing such a success (survival Chapter 9 and growth Chapter 7) critically depends on returned fish (Chick, 2010). The objective of this Chapter was to compare several tagging techniques in order to select a reliable and consistent method that allowed us to identify the release meagre when recaptured from the wild by the fishermen after an extended period. Different aspects have been analysed (Table 4.5) in order to determine the pros and cons of each tested tag.

	T-bar	Internal	VIE	ALR	OTC
Ease to identify by the fishermen	Yes	Yes	No	No	No
Identification of fish	Individual	Individual	Cohort or group	Cohort or group	Cohort or group
Retention rate	Medium-low	High	Low	High	Unsuccessfull
Mortality	1.6[1]	20.6[1]	-	7.5%	7.7%
Growth	No effect	No effect	No effect	-	-
Cost	0.67€/tag	1.021 €/tag	0.14€/tag	0.08€/fish[2]	0.02€/fish[2]
Marking time	3 seconds	2-3 min[3]	2-3 min[3]	22 hours	21 hours
Bath marking	No	No	No	Yes	Yes

Table 4.5.- Pros and cons of the tested tagging methods on *A. regius* juveniles. [1] Short term mortality. [2] For a bath of 2 months old meagres (n = 1000) with a density of 2 kg m^{-3}. [3]This method requires that the fish be anesthetized (included in the marking time).

The T-bar anchor tag is widely used because it is quickly and easily applicable, has a relative low cost and negligible mortality. However this tag type showed a low retention time in juvenile meagres. The smallest fish tested (age-0+) presented a very low retention rate, reaching 0% at 208 days after tagging date. Retention rate for larger fish (age-1+) seems to improve but is still suboptimal, reaching 70% of tag after the same time. This great difference could be in part explained by the fact that the tags used in these two trials were not exactly the same. In some cases, we observed that the anchor of the smallest tags (FF-94) were broken (thus, the ID code was lost), while this was never observed in the largest (FD-94) tags. This fact was also reported by Otterå *et al.* (1998) with a small anchor T-tag. Moreover, Davis and Reid (1982) also found that using short needles for the tag pistol seems to increase the rate of tag loosing. A comparative review showed variable retention rates (Booth & Weyl, 2008). For example, after 1 year, anchor tag retention was 93% for Arctic grayling (Buzby & Deegan, 1999) and 89% for lake whitefish, *Coregonus clupeaformis* (Ebener & Copes, 1982). While, anchor tag retention was 42% after 1 year for striped bass (Waldman *et al.*, 1991), 25% after 560 days for white bass, *Morone chrysops* (Muoneke, 1992) and 9% after 198 days for red Roman, *Chrysoblephus laticeps* (Kerwath *et al.*, 2006). Most of the reported differences in tag loss rates are probably due to different tagging methods, differences between species, differences between the fish size and differences between the persons that did the tagging (Otterå *et al.*, 1998). Therefore, tag retention rate should be individually evaluated for each species, each fish size and each model of tag.

In this study, diet and temperature comparisons showed significantly lower T-bar tag retention for fish well fed and submitted to higher temperatures. The tag loss could be related with the scrape against hard structures or the high fish densities (Sánchez-Lamadrid, 2001; Booth & Weyl, 2008). Therefore an optimal nutritional condition and high temperatures may result in increased activity favoring tag loss. The density was not exactly the same for all the tank experiments but was low enough to not interfere comparisons. However an increased activity can result in greater interaction between the fish. Released meagres need a relatively long time to adapt to the wild food (Chapter 6). Therefore, short term studies do not provide reliable estimates of tag retention at the long term. Nevertheless, returned fish that have spent long time at liberty seems to show high rate of tag loss. In contrast, short term tag loss seems to be smaller, which may be related with suboptimal diet of recently released fish, which may be less active than fully feed fish, as suggested by the tank experiments. However, the tag loss rate estimated at these experiments should be extrapolated to the wild with caution because the characteristics of the natural environment and the fish's behaviour in the wild could modify them. Therefore, tag retention studies should be conducted in natural conditions (Booth & Weyl, 2008).

Very high retention rate were observed for the internal anchor tag (Table 4.5). Similarly Wallin *et al.* (1997) reported a retention higher than 99% after 30 days for common snook, *Centropomus undecimalis* and Dunning *et al.* (1987) of 98% after 1 year for striped bass, *Morone saxatilis*. The insertion of the internal anchor tag is a process by far more traumatic than the insertion of the T-bar anchor tag because it need a large incision in the abdominal wall and requires anesthesia. This fact was reflected in the case of meagre in a high mortality. However, in previous studies this difference was not observed (Dunning *et al.*, 1987; Wallin *et al.*, 1997). Although, irritation and haemorrhage, as well as secondary infections around the insertion site has been reported (Vogelbein & Overstreet, 1987; Phelps & Rodriguez, 2011). However a successful healing and no-infection were observed in the fish tagged with internal anchor tag that survive in the tank experiment (Fig. 4.8).

Fig. 4.8.- *A. regius* with internal anchor tag as seen 125 days post-tagging.

Contrary to what was expected, VIE presented a low retention rate in this study, while high retention has been reported elsewhere (Catalano *et al.*, 2001; Brennan *et al.*, 2005; Kerwath *et al.*, 2006). It has been noticed that VIE tends to fragment over time and thus may became difficult to see (Astorga *et al.*, 2005), but this was not the problem in this study because VIE began to disappear few weeks after release. In the case of VIE, body location, the experience and ability of the personal and the amount of resin that is injected can significantly influence tag retention and visibility; therefore, further research could improve the obtained results. Nevertheless, the preliminary results reported here do not advice the use of this method.

Concerning OTC, bathing in marine water was demonstrated to be ineffective for meagre. Taylor *et al.* (2005) showed for *A. japonicus* that OTC available for uptake decreases with salinity and is negligible at 35‰ regardless of the concentration of OTC provided. Therefore the lack of OTC mark in the meagre otoliths in the 2006 experiment might have been due to a reduced availability of the OTC to the fish because the marking tank salinity was 37‰. Meagre is an ideal species for testing whether reducing salinity improves OTC marking success because small-sized juveniles are commonly found inhabiting brackish waters in estuaries and salt marshes (Baldó & Drake, 2002). However, the experiment performed in October 2008, showed that *A. regius* is more affected by abrupt reductions of salinity than *A. japonicus*, and this sensitivity was related to age, being stronger for older meagres. These constraints make batch OTC marking impracticable at low salinity, and thus precludes the use of this method for meagre.

ALR was a very efficient marker in meagre, irrespective of age at marking and has very low mortality associated to the treatment. Success (fish with marks at the otolith) surpasses 90%. ALR marks have been detected up to 3 years after immersion (Tsukamoto *et al.*, 1989; Reinert *et al.*, 1998; Jenkins *et al.*, 2002).

All the tested tag methods have some pros and some cons (Table 4.5). OTC and VIE have a low cost but are ineffective for tagging of meagre. ALR have a high retention, low mortality and low cost when it is used in small fish (a large number of small fish can be marked at the same time). However, this mark is not visible by fishermen and so we loose relevant pieces of information that are needed for evaluating the success of the restocking program. Internal anchor tags have a high retention and are visible by fishermen but they presented a high mortality in the tank experiment. Therefore this method is clearly suitable for meagre. T-bar anchor tags have a quick and easy application and a low cost, allowing us to tag a large number of meagres in a short time. Besides, they are visible by the fishermen who provide individual information of the recaptured meagres, allowing to carry out detailed studies about movement, growth, feeding or survival. However, there are evidences, both at the wild and in tank experiments suggesting that the T-bar tags has an important loss rate over the time, which may introduce some biases and complexities when analyzing the data obtained in this way (but see Chapter 9 for an unbiased method for survival estimation that is robust to non reporting rate).

In conclusion, to ensure sufficient data collection for the meagre study case, we propose and apply a double-tagging approach: T-bar anchor tag and ALR bath was applied in all the meagre juveniles released into the wild. Thereby, short-term detailed information was obtained with the T-bar tag and, through the ALR otolith mark, large-term information may be still obtained when fishermen return large and untagged meagres or when they are bought at the fish market. A compromise solution is to restrict the number of release event to one per year or to apply more than one bath to the same fish in order to get a specific “bar code” at the otolith. In this way, any returned fish can be unambiguously attributed to a specific release event even when the T-tag is lost because the age of the fish can be estimated from the otolith. An efficient alert system coupled to the fish auction has been implemented to obtain more information of the recaptures of these large fish (Chapter 9).

SECTION V – Monitoring meagre recaptures

Recaptured meagre by a professional fishermen (Photo: Oliver Navarro)

Chapter 5. Dispersion and movement pattern of released juveniles

Surgically implantation of acoustic transmitter in meagre.

Dispersion and movement pattern of released meagre juveniles

Abstract

The dispersion and the movement pattern of the released hatchery-reared meagres into the wild were examined using data storage tags, acoustic telemetry and mark recapture data. Three out the 19 fish implanted with data storage tags were recovered. The depth data recorded showed a clear diel pattern suggesting high activity at night when records within a wide range of depth are monitored, and low activity during day-time, when fish seem to remain at the same depth. Almost all of the 30 fish implanted with acoustic transmitters were detected a relatively low number of times. In addition, most of the fish were detected by only one or a few receivers, indicating that fish spent short time within the area covered by the array of acoustic receivers, thus suggesting high mobility. The few fish detected by more than 5 receivers seem to follow a random-walk type of movement, with acute changes of direction between time steps. Finally, recapture date and locations of a large number of recaptured meagres (383 fish) were used to analyze after release dispersion. Dispersion capability of meagre was noteworthy, being able of moving a maximum of 149.6 km apart in only 12 days. Overall, the data obtained suggest that meagre would move randomly but speedy at night-time but come back to low depth sites at day-time, when fish would be attached to a fixed bottom site.

5.1 Introduction

The individual movements and the temporal and spatial scales of fish movement throughout the lifecycle are fundamental determinants of population dynamics and genetic structure, although there is a wide range of movement patterns along the life history, and movement is heterogeneous both within and among individuals (Morrissey & Ferguson, 2011). The success of restocking programs depends on manifold aspects of the life history and the ecology of the target species but accurate knowledge of the movement pattern is one of the most important (Rakes *et al.*, 1999). Post-release movement and diel activity patterns are closely related to mortality, which mainly depends on fishing effort and natural predation, both processes directly affecting the recapture rate of the fish (Kawabata *et al.*, 2007; Kawabata *et al.*, 2011). Movement traits such as agonistic behaviour, migration, homing, foraging behaviour and use of cover may be either lacking or altered in fish reared at a hatchery environment (Weiss & Kummer, 1999). Fish produced for restocking are usually reared in simple tanks without any of the complexities of wild habitats. Differences in movement pattern between wild and hatchery-reared specimens have been observed in several studies (Weiss & Kummer, 1999; Teixeira *et al.*, 2006), suggesting that fish must learn to behave properly at an *a priori* hostile environment until they adapt to the natural habitat (Ellis *et al.*, 1998; Ireland *et al.*, 2002). Learning includes, for example, responding to the appropriate cues for initiating migratory behaviour or being able to locate feeding and spawning habitats (Grabowski & Jennings, 2009). Hatchery-reared individuals also tend to exhibit much higher activity levels immediately after stocking than after they have spent some time at liberty. This behavioural pattern may leads to the dispersal of a significant proportion of sexually immature stocked individuals out of the population they were meant to enhance (Cresswell, 1981; Mueller *et al.*, 2003).

As it has been detailed in the Chapter 1, wild adult meagres migrate to estuarine and coastal areas to reproduce (Quéro & Vayne, 1987; Griffiths & Heemstra, 1995; Quéméner, 2002; Cárdenas, 2010). Larval and early-juvenile stages remain near the spawning area. Juvenile meagres (*ca*. 20-70 cm L_T and 0^+-3 years) may progressively expand their habitat in coastal waters between 5-40 m of depth (Quéro, 1989; Catalán *et al.*, 2006b). However, there is lack or scarce information of the post-release dispersal movements and on the existence of any diel activity pattern of either, hatchery-reared meagres or wild fish.

Mark recapture methods have been used to study three main processes. Namely, movement patterns of individual fish, population dynamics and fish growth (Stevens, 1976, 1990; Casey & Kohler, 1992; Kohler & Turner, 2001; Voegeli *et al.*, 2001). Recent advances in technologies such as satellite and Global Positioning System (GPS) transmitters, acoustic telemetry and electronic data storage tags have led to a vast influx of data on the movement of tagged individuals from a diverse range of species in habitats where direct observation is difficult or even impossible (Patterson *et al.*, 2008). However, each observation method has pros and cons. Mark recapture method allows tagging a large number of fish quickly and cheaply, but data obtained may be biased by the sampling schemes (e.g., locations and/or timing of reported recaptures by the commercial fishery might reflect fisher behaviour rather than fish behaviour) or by behavioural responses to some specific fishing gears (Smith *et al.*, 2000; Devineau *et al.*, 2006; Patterson *et al.*, 2008). Satellite transmitters provide accurate position information over large areas, but are only effective in those animals that allow sufficient contact with earth-orbiting satellites for both, data collection and location determination, such as sharks, marine mammals or sea turtles (Eckert & Stewart, 2001; Bentivegna, 2002). Acoustic telemetry is used, for example, (1) to quantify home range size (Lowe *et al.*, 2003; Parsons & Egli, 2005; Alós *et al.*, 2011), (2) to determine site fidelity (Collins *et al.*, 2007; Abecasis & Erzini, 2008; Abecasis *et al.*, 2009; Semmens *et al.*, 2010) and (3) to describe temporal patterns, such as diel changes (Jadot *et al.*, 2006; Jorgensen *et al.*, 2006; March *et al.*, 2010; Cabanellas-Reboredo *et al.*, 2012). However, telemetry suffers from substantial positional imprecision (Patterson *et al.*, 2008) and is limited to constrained areas. Data storage tags allow to get long term and high resolution data on vertical distribution to identify any diel rhythm (Godø & Michalsen, 2000), but their efficiency depends on the recapture rate of the study species, which is usually low (Oxenford *et al.*, 2003; Sepulveda *et al.*, 2011).

The main objective of this Chapter was to provide information on the temporal and spatial use of wild habitat by released juvenile meagres. Dispersal capability, movement characteristics and diel activity pattern were described for first time by combining evidences obtained with acoustic telemetry, data storage tags and mark recapture methods.

5.2 Materials and Methods

Three different methods were applied for the study of the movement and distribution of the released meagre juveniles: data storage tags, acoustic telemetry and mark recapture data.

5.2.1 Data storage tags experiment

Two releases of fish with electronic data storage tags were performed. The first release was done in July 2011 and the second one in January-February 2012. In the first release, 10 data storage tags were used to tag 11 juvenile meagres because one of them was recaptured after few days and the same tag was reused in another fish. In the second release, 6 data storage tags were used to tag 8 juvenile meagres because 2 of them were recaptured (Table 5.1).

ID	Surgery data	Release data	Age (months)	L_T	W_T
12745	30/06/2011	01/07/2011	26	50.5	1160
12746*	30/06/2011	01/07/2011	26	54	1498
12737	30/06/2011	01/07/2011	26	48.6	951
12734	30/06/2011	01/07/2011	26	45.1	867
12740	30/06/2011	01/07/2011	26	46.5	861
12743	30/06/2011	01/07/2011	26	43	768
12747	30/06/2011	01/07/2011	26	57.4	1215
12751	30/06/2011	01/07/2011	26	47.9	1001
12742	30/06/2011	01/07/2011	26	47.5	1026
12749	04/07/2011	05/07/2011	26	42.8	712.7
12746	13/07/2011	15/07/2011	26	48.2	1036.1
13212	11/01/2012	12/01/2012	32	48.6	1160.7
13208	11/01/2012	12/01/2012	32	47.1	922.2
13199	11/01/2012	12/01/2012	32	49.9	1245.8
13209*	11/01/2012	12/01/2012	32	50.2	1255.9
13200	11/01/2012	12/01/2012	32	50.7	1358.8
13211	11/01/2012	12/01/2012	32	44.5	937.4
13209*	26/01/2012	27/01/2012	58	70	3606
13209	28/02/2012	29/02/2012	59	75.5	3958

Table 5.1.- Meagre juveniles with implanted data storage tags.

The used data storage tags were Star-Oddi DST milli-TD (Star-Oddi, Reykjavik, Iceland; Fig. 5.1). These tags were 38.4 mm in length, 13 mm in diameter, and 5 g in weight under water. The storage capacity was 43000 records for the tags used in 2011, and twice this capacity for the tags used in 2012. The temperature range within which the sensors were operative was from -1 ºC to +40 ºC, and the depth range was from 10 cm to 100 m. The resolution of depth estimated for these DST milli tags was 0.03% of the range (Star Oddi, Iceland).

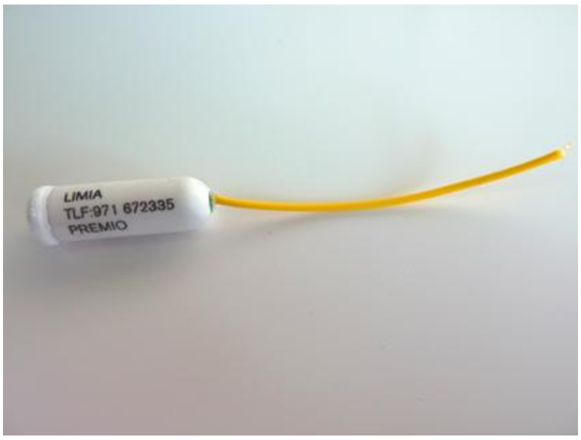


Fig. 5.1.- Data storage tag used on juvenile meagres. The yellow tube remains externally to make the tag visible by the fishermen.

The tags were programmed to record depth and temperature measurements in a way that balance between short and long term information was optimized (Table 5.2).

Year	Duration period	Deteccion periodicity	Num. Detections
2011	5 days	2 minutes	3600
	85 days	1 hour	2040
	5 days	2 minutes	3600
	85 days	1 hour	2040
	5 days	2 minutes	3600
	175 days	1 hour	4200
	36 months	12 hours	2160
2012	5 days	1 minute	7200
	85 days	30 minutes	4080
	5 days	1 minute	7200
	85 days	30 minutes	4080
	5 days	1 minute	7200
	175 days	30 minutes	8400
	36 months	6 hours	4320

Table 5.2.- Programmed periods for the data storage tags used in 2011 and 2012. The number of detection is expressed for each parameter.

Star-Oddi DSTs were surgically implanted into the peritoneal cavity of meagres anaesthetized with a solution (100 mg L^{-1}) of tricaine methanesulfonate (MS-222®), one of the most widely used anaesthetics for poikilotherms worldwide. The incision was closed using polyglactin 910 sutures (Vicryl® Rapid 5-0, an absorbable, synthetic, braided suture). After surgery, fish were kept for at least one day under controlled conditions to ensure full recovery.

Plots of depth and temperature over time were made for each recaptured specimen.

5.2.2 Acoustic tracking experiment

5.2.2.1 Experiment 2009

The first acoustic tracking experiment was carried out in May 2009 at Palma Bay Marine Reserve, Balearic Islands (NW Mediterranean), with one year old meagre juveniles (L_T: 24.0 ± 1.2 cm and W_T: 142.4 ± 26.0 g). A total of 105 meagres were released, of which just 10 were equipped with implanted radio telemetry transmitters. This releasing strategy was adopted because juvenile and sub-adult *A. regius* form schools (Chao, 1986; Quéro & Vayne, 1987; Quéro, 1989; Quéméner, 2002; Catalán *et al.*, 2006a), thus isolated fish may behave in a different way. All fish were measured, weighed and tagged with external T-bar anchor tags (Floy Tags©). The specimens chosen for telemetry were anaesthetized with MS-222® (as detailed above). Then, the transmitters were surgically inserted into the peritoneal cavity through an incision made with a scalpel along the ventral midline midway between the pelvic fins and the anus. The incision was closed using Vicryl® Rapid sutures. The acoustic tags used were Sonotronics® IBT-96-1-I (Fig. 5.2a), which had a size of 25mm x 8 mm, a weigh in water of 1.5 g and a battery life of 21 days. These transmitters did not exceed 1.7% of the body weight of the fish. The fish were allowed to recover for one day before release. Well recovered fish were released at 3 different points within the receivers array (see below).

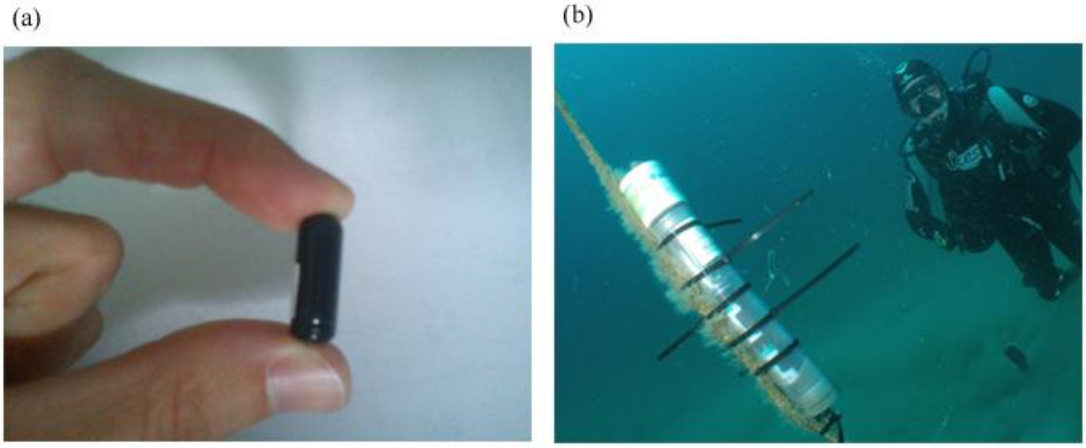


Fig. 5.2.- (a) Acoustic tag IBT-96-1-I used in the experiment 2009. (b) Receiver (Sonotronics, SUR-1) fixed to a rope.

The acoustic monitoring system (Sonotronics, SUR-1) used small multifrequency, omnidirectional receivers (Fig. 5.2b; 368 mm in length, 60 mm in diameter, 766 g, with small positive buoyancy on water and 10 months of battery life), that scanned up to 15 frequencies (from 69 kHz to 83 kHz), measuring and recording the time intervals between the successive beeps emitted by the transmitters. Each transmitter had a specific combination of beeps and silences that allowed its identification. The receivers were oriented upwards on a rope to 1-2 m from the bottom. The rope was moored with a cement block, and with a sub-surface buoy located between 5 and 8 m in depth.

An array of 17 receivers was deployed at the Palma Bay Marine Reserve (Fig. 5.3). The receivers covered an area of approximately 7.5 km^2 and the averaged distance between them was ~900 m. This array was designed to study the previously unknown spatial range of 1 year old meagre, which was expected to move fast and cope a wide area.

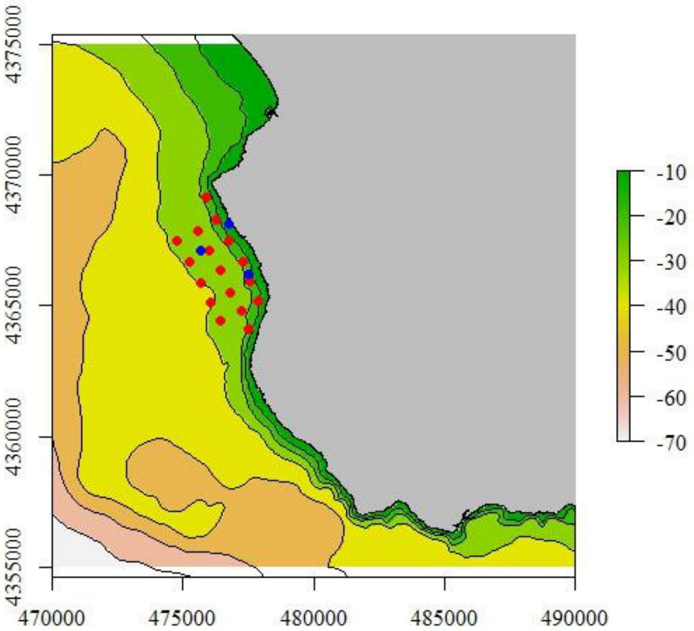


Fig. 5.3.- Map of the study area showing the location of the acoustic receivers (red points) in the Palma Bay Marine Reserve. The blue points indicate the places where meagres were released.

After the expected battery life time of the transmitters was over (21 days), the receivers array was retrieved, and the data were downloaded from each receiver. Any single detection event was labelled with an ID code, date (mm/dd/ yyyy), hour (hh:mm:ss), frequency (kHz), and interval period (ms). A tolerance interval of 5 ms was selected for detecting and removing putative false detections (i.e., spurious detection related with noise at the same frequency but beeps having a different periodicity), following the conservative criteria adopted by other studies that used the same tracking equipment in the same area (March *et al.*, 2010; Alós *et al.*, 2011; March *et al.*, 2011; Alós *et al.*, 2012; Cabanellas-Reboredo *et al.*, 2012).

5.2.2.2 Experiment 2010

The second acoustic experiment was carried out between March and July 2010, with 2 year old and 4 year old meagres. Ten meagres born in 2008 (L_T: 40.7 ± 2.3 cm and W_T: 699.3 ± 153.7 g) and 10 meagres born in 2006 (L_T: 64.5 ± 4.9 cm and W_T: 2634.8 ± 671.6 g) were tagged. Previously all fish were measured, weighed and tagged with external T-bar anchor tags, and then the transmitters were implanted following the same procedure detailed above for the experiment 2009. The acoustic tags used in this experiment were Sonotronics® CT-82-2-I (Fig. 5.4), which had a size of 53 mm x 15.6 mm, a weigh in water of 9.5 g and a battery life of 14 months.

Fig. 5.4.- Acoustic tag CT-82-2-I used in the experiment 2010.

An array of 17 receivers was more sparsely distributed in the southern waters of Mallorca Island and one additional but isolated receiver was deployed in the north coast (Fig. 5.5a), in order to detect eventual large movements made by meagre. The distances between the receivers ranged from 2.6 to 8.9 km (except for the isolated receiver in the north coast). In July, the receivers array was retrieved and the data were downloaded following the same procedure detailed for the experiment 2009.

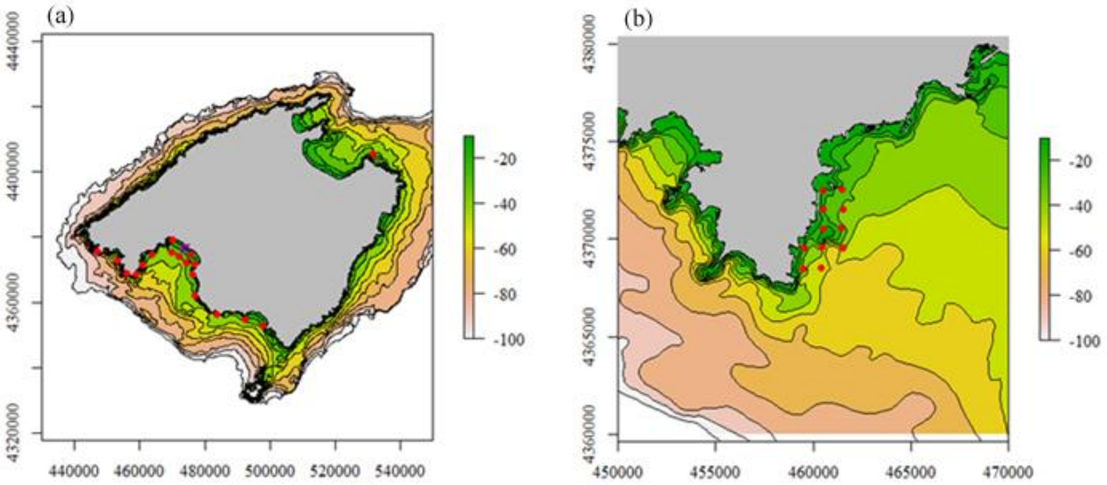


Fig. 5.5.- (a) Map of the study area showing the location of the acoustic receivers (red points) in the experiment 2010. (b) Map of the study area in Alós *et al*., (2012) and the location of the acoustic receivers.

One of the fish tagged in this experiment was also detected by a third array of receivers deployed for another study (Alós *et al.*, 2012). This array was constituted by 11 receivers located in southwestern Palma Bay (Fig. 5.5b) from September to December 2010.

5.2.3 Mark recapture data

All the released meagres for restocking (see Appendix Table I.1) were tagged with T-bar anchor tags (Floy Tags©) previously to be released at specific places and dates (see Appendix Figure I.2).

When a tagged meagre was recaptured by professional and recreational fishermen, an interview was carried out to determine, as accurately as possible, the recapture date and location. For a few cases, only imprecise description of the recapture location was available, or the release date and location could not be known due to tag loss and the unavailability of otoliths. These cases were not included in the analyses below.

5.2.4 Data analyses

To estimate true swimming speed is challenging because fish trajectory may be more or less tortuous and the available data consist only in straight line distance travelled by time unit. However, we estimated *maximum swimming speed* at two very different spatial (and temporal) scales with the specific objective of describing such a tortuosity.

At the short-term scale (acoustic tracking), maximum swimming speed (km h^{-1}) was estimated for each fish by dividing the distance movement between any two consecutive detections made by two different receivers by the time elapsed. When receivers were located very close each other, it was possible that the same beep was detected by more than one receiver. Moreover, it should be noted that the raw data of acoustic tracking consists of a collection of positions of the receivers that detected the fish but the fish positions themselves remain unknown. Detection probability is distance dependent. The radius within which the probability of detection is larger than 0.5 has been empirically estimated, in our case, low than 150 m (Cabanellas-Reboredo *et al.*, 2012). This positioning error has no practical consequences when between-receiver distances are much larger that detection radius, but in other cases like those of experiment 2009, it was possible that a fish may have been located just in the middle of two receivers and, thus, it may have been detected almost simultaneously by them. The speed measured in this way would have been clearly overestimated if it was assumed that the fish moved from one receiver to the other. Therefore, the raw data used for estimating maximum swimming speed was filtered and consecutive detections made by receivers located at less than ~900 m were conservatively excluded (i.e., in the experiment 2009, the detections between each receiver with the most close receivers were deleted).

At the long-term scale, maximum swimming speed was also estimated for the mark recapture data. In this case, the distance travelled was estimated as the minimum distance between the release and the recapture site (i.e., avoiding land crossing), and the time elapsed was the days between the release and the recapture date.

5.3 Results

5.3.1 Diel activity pattern

Three data storage tags were recovered after spent 2, 8 and 24 days at liberty (Table 5.3), and their recorded data could be downloaded.

ID	L_T (cm)	Release date	Recapture date	DAL	Num. records
12746	54	01/07/2011	03/07/2011	2	1640
13209	50.2	12/01/2012	20/01/2012	8	7348
13209B	70	27/01/2012	20/02/2012	24	8114

Table 5.3.- Summary of the recaptured meagres with implanted data storage tag.

The plot of the measurement of each recaptured tag showed substantial vertical activity (Fig. 5.6). There were two basic patterns in the depth data: 1) periods of very limited vertical movements, and 2) periods of relatively frequent vertical movements. Almost all the vertical movements occurred at night and during the day meagres seem to remain at constant or nearly constant depth, except at the release date when the meagres performed important vertical movement at day-time. The temperature pattern only corresponded with the depth pattern in the specimen released in summer, where relatively sharp temperature decrease was observed in depth higher than 18 m. However, the temperature pattern was almost constant in the other specimens released in winter due to water mixing, and the small changes detected were not correlated with depth changes.

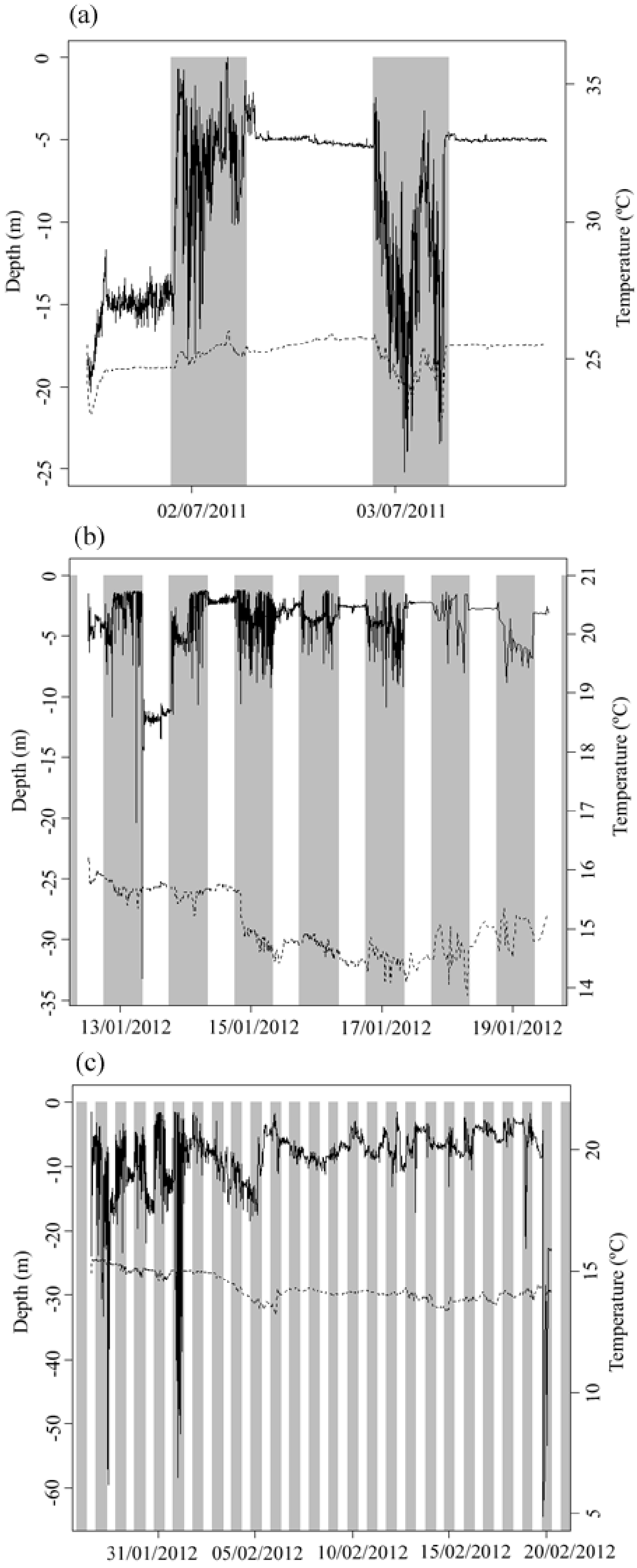


Fig. 5.6.- Depth (continued line) and temperature (discontinued line) recordings of recaptured meagres with implanted data storage tags. (a) Fish 12746, (b) fish 13209 and (c) fish 13209B. The vertical stripes represent day (white) and night (grey).

The depth recorded during the day-time was commonly lower than 20 m (Fig. 5.7a). Instead, at night fish reached higher depth up to 64.3 m but the most common depth was also lower than 20 m (Fig. 5.7b).

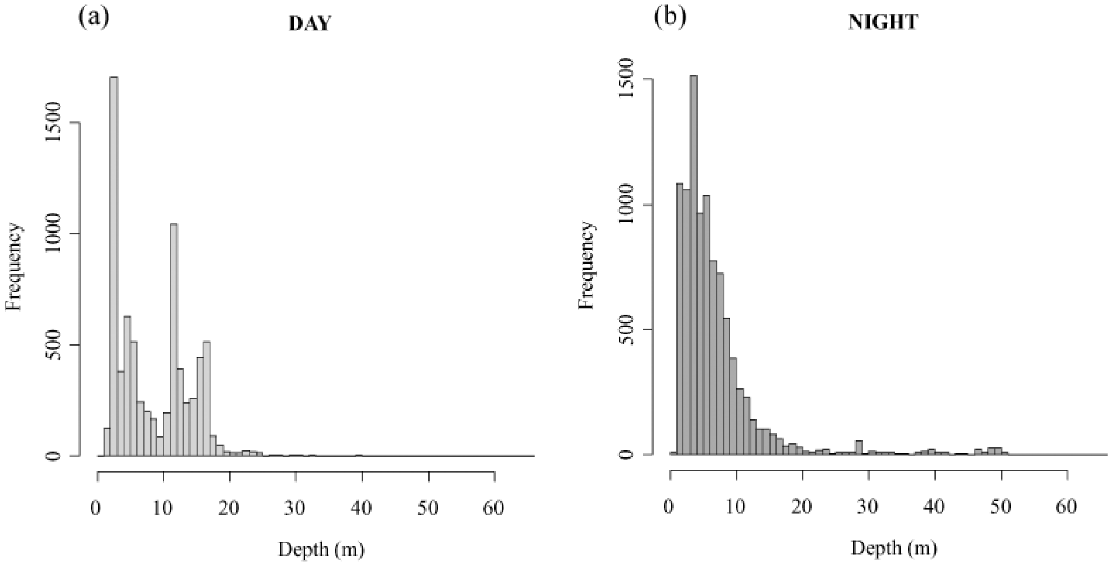


Fig. 5.7.- Frequency histogram of depth for the recaptured meagres with implanted data storage tags (a) during the day and (b) at night.

5.3.2 Movement

A total of 1138 detections were downloaded from the receivers (Table 5.4). During the experiment 2009, 2 fish were not detected, whereas only one fish tagged with acoustic transmitter in the experiment 2010 was not detected. Nearly half of the detections (524) were emitted by the same fish (#79). On the other side, 5 fish were detected only once. The tagged fish were detected by different number of acoustic receivers, ranging from 1 to 10. The fish were tracked over time periods from 1 day to 227 days (Fish #66 was detected some time later by another array; see below). However, during this detection period, the number of days a tagged fish was within the acoustic array was low and fish were detected up to 7 days.

Fish ID	Experiment	Released	L_T (cm)	Release date	TP (d)	DD (d)	Total detections	Num. of receivers
50	2009	Site 1	23.5	13/05/2009	1	1	5	2
51	2009	Site 1	23.2	13/05/2009	16	3	48	8
53	2009	Site 1	25.1	13/05/2009	4	3	25	2
47	2009	Site 2	23.8	13/05/2009	-	-	-	-
48	2009	Site 2	22.2	13/05/2009	4	4	105	10
49	2009	Site 2	25.6	13/05/2009	-	-	-	-
52	2009	Site 3	23.2	20/05/2009	1	1	1	1
54	2009	Site 3	23.0	20/05/2009	4	4	16	6
55	2009	Site 3	26.3	20/05/2009	2	2	16	7
56	2009	Site 3	25.6	20/05/2009	1	1	1	1
61	2010	Meagre 2006	66.2	15/04/2010	12	1	1	1
72	2010	Meagre 2006	65.8	15/04/2010	49	7	19	3
73	2010	Meagre 2006	60.5	15/04/2010	5	2	27	3
74	2010	Meagre 2006	65.8	15/04/2010	19	1	24	1
75	2010	Meagre 2006	63.2	15/04/2010	1	1	1	1
77	2010	Meagre 2006	53.5	15/04/2010	3	2	5	2
78	2010	Meagre 2006	67.2	15/04/2010	50	3	24	7
79	2010	Meagre 2006	64.2	15/04/2010	7	5	524	2
81	2010	Meagre 2006	72.8	15/04/2010	-	-	-	-
82	2010	Meagre 2006	66.0	15/04/2010	2	1	5	1
62	2010	Meagre 2008	42.9	31/03/2010	11	2	12	3
63	2010	Meagre 2008	41.0	31/03/2010	1	1	4	1
64	2010	Meagre 2008	44.3	31/03/2010	1	1	1	1
66	2010	Meagre 2008	41.5	31/03/2010	227	4	111	9
67	2010	Meagre 2008	43.7	31/03/2010	2	2	27	1
68	2010	Meagre 2008	41.3	31/03/2010	3	3	31	1
69	2010	Meagre 2008	38.7	31/03/2010	9	2	23	2
70	2010	Meagre 2008	41.6	31/03/2010	1	1	16	1
71	2010	Meagre 2008	44.2	31/03/2010	13	2	6	2
80	2010	Meagre 2008	39.9	31/03/2010	15	4	58	2

Table 5.4.- Summary of the monitoring data for the 30 tagged *Argyrosomus regius*. L_T, total length; TP, total time period between the release date and the last detection; and DD, number of days detected. The total numbers detections as well as the number of acoustic receivers that detected the fish are also presented.

Most of the tagged fish (Fig. 5.8) were detected by a low number of acoustic receivers and the number of detections was low in almost all the cases. Overall, these data indicates that fish tend to move most of the time outside the detection rage of the acoustic array.

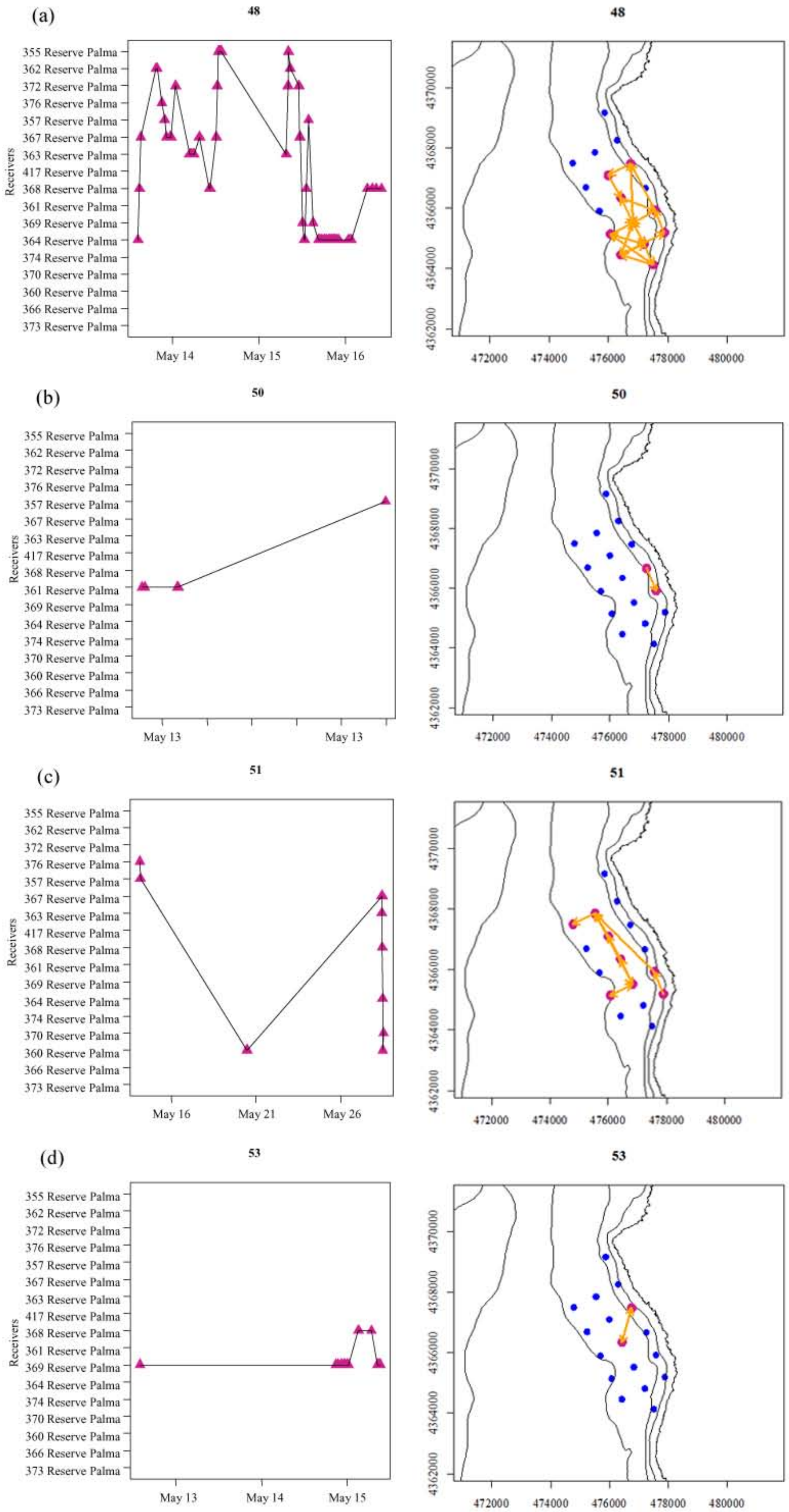


Fig. 5.8.- Detections of all the meagres detected by more than one receiver. In the left side, receivers that detect each fish over time. In the right side, meagre tracks assuming the minimum distance travelled. Note that the temporal scale is different for each fish.

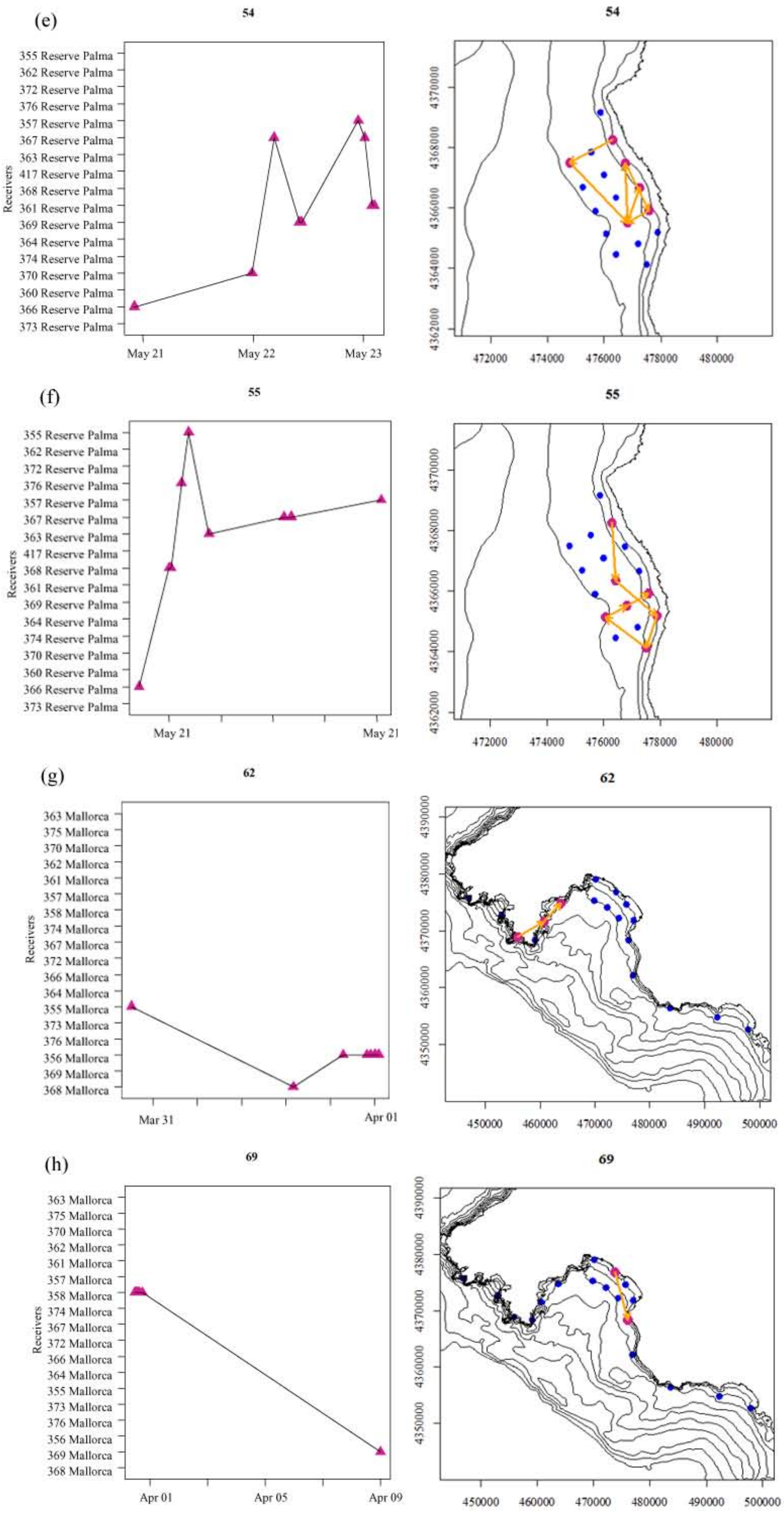

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Fig. 5.8 (cont.).-

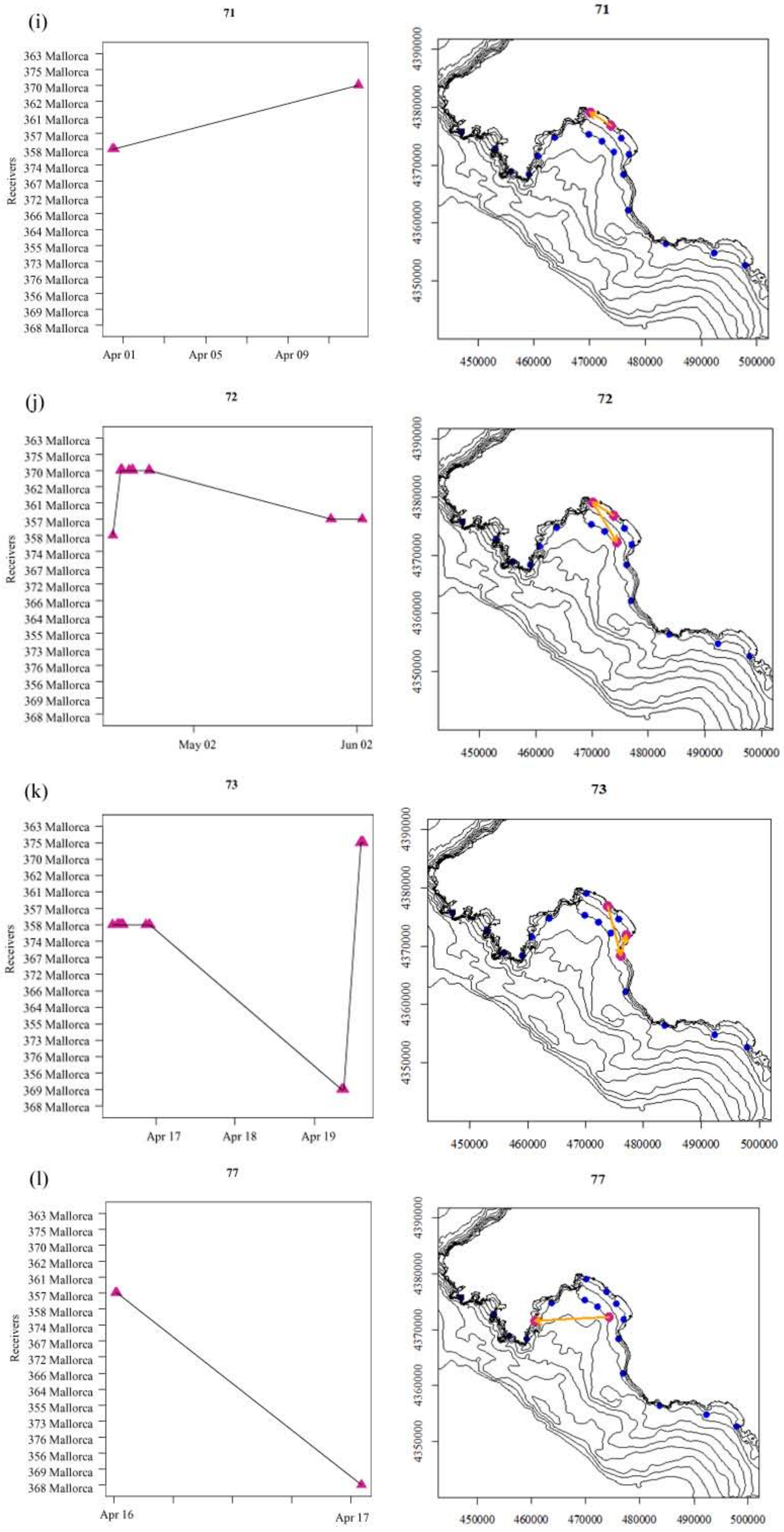
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Fig. 5.8(cont.).-

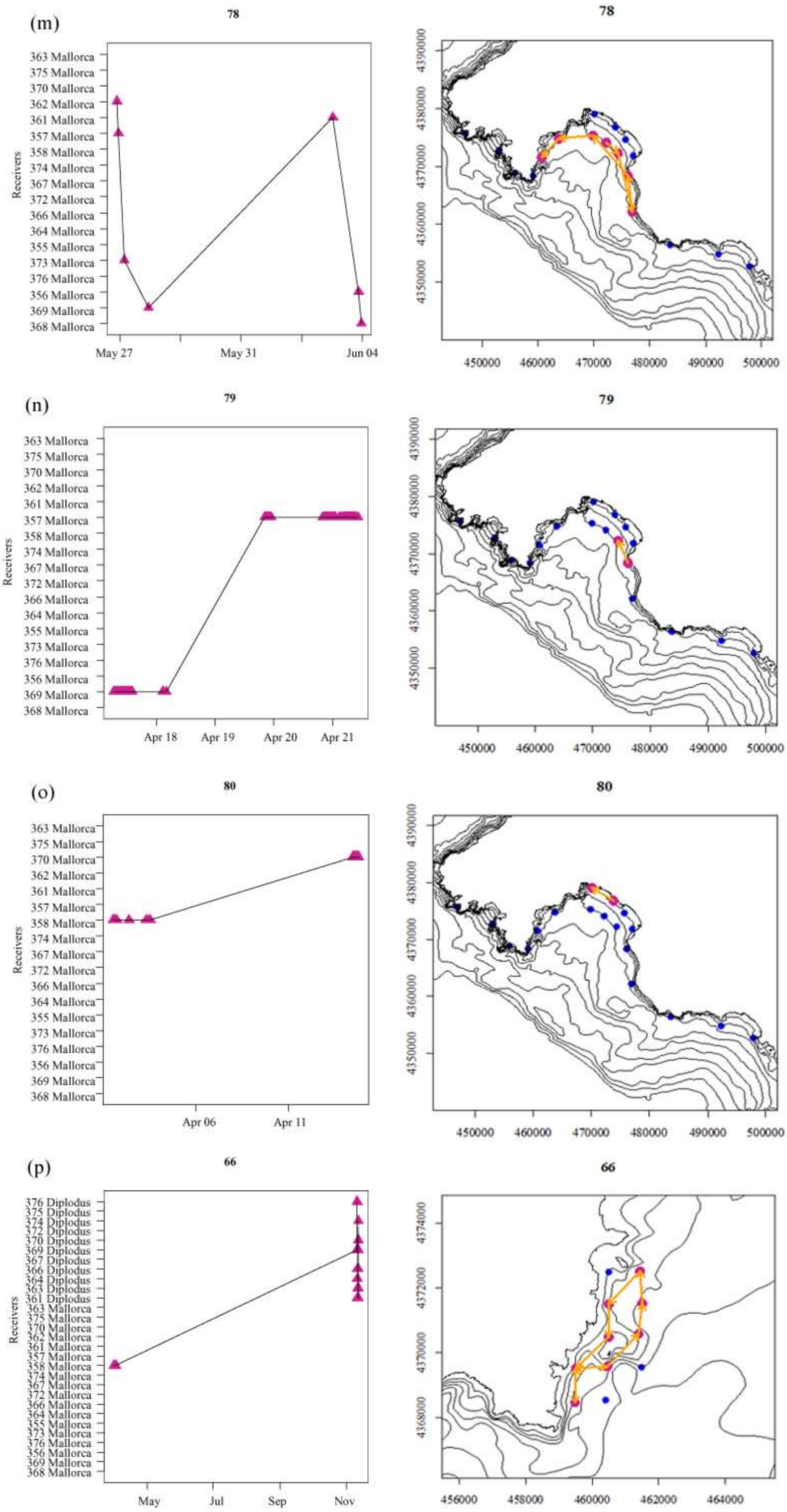
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Fig. 5.8(cont.).-

However, some of the tagged fish (Fig. 5.8a,c,e,f,m,p) were detected by a large number of receivers in a short time. Therefore, these fish showed not only a great movement capability but also that fish continuously changed movement direction, thus suggesting a random walk type of movement, or at least a movement without a clearly defined displacement direction.

5.3.3 Swimming speed

The detections obtained from the different receivers allowed estimating the swimming activity from the time elapsed and minimum distance travelled between any two consecutive positions. As fish did not swim directly from one receiver to other, the estimated swimming speed showed a wide range of values (Fig. 5.9) with a large number of values close to zero. This large amount of low value was plausibly related with fish moving erratically, thus travelling distances much longer than the straight line between two consecutive positions. However, it is expected that the highest estimates of swimming speed would asymptotically approximates to the swimming speed of the released meagres at a short spatial-temporal scale. The highest observed swimming speed was 3.0 km h^{-1} for the Fish #48 (Fig. 5.9a).

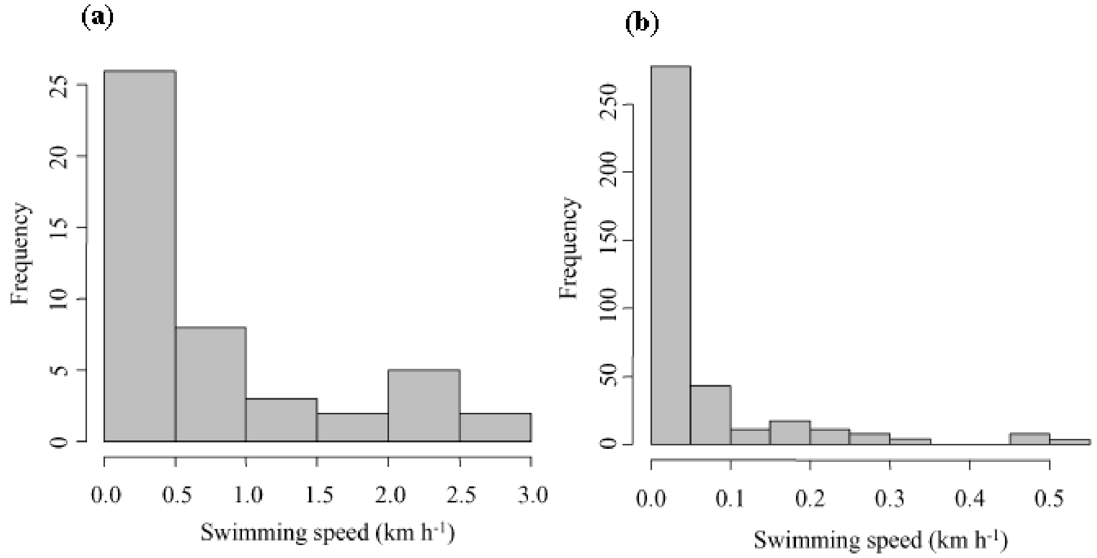


Fig. 5.9.- Frequency histogram of swimming speed for (a) meagres tracked with acoustic telemetry and (b) meagres from mark recapture data.

As expected, swimming speed obtained for the mark recapture meagres (Fig. 5.9b) was much lower than the values estimated for acoustically tracked fish. The maximum estimated value was 0.54 km h^{-1} for two fish that travelled from the release location

(Andratx, see Appendix Fig. I.2) to Pollença, that is, a minimum distance of 90.6 km in 7 days.

5.3.4 Post-release dispersal

From all recaptured meagres (see Appendix Table I.3), release and recapture information (date and location) was accurate for 383 (89.2%) fish. Focusing in those fish, the time elapsed between release and recapture ranged between 1 and 1560 days, while the distance travelled ranged between 115 m and 149.6 km. This maximum distance was covered in 12 days. The distance travelled between release and recapture was very variable even for fish that had spent short time at liberty (Fig. 5.10). Focusing only on fish that had spent 50 days or less at liberty, 95% of them dispersed at distances smaller than 56.6 km but it is remarkable that the remaining 5% travelled longer distances.

After more than one year at liberty, meagres were recaptured both close and far from the release location. It is especially remarkable that after such a time, any point around the coastal area of the island could be reached by a released meagre.

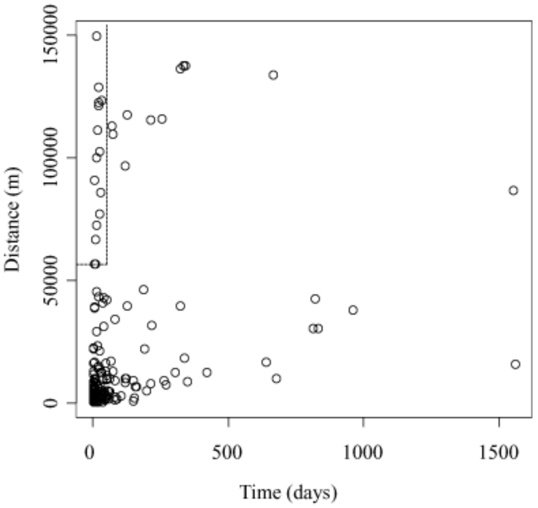


Fig. 5.10.- Observed relationship between the time at liberty and the distance between the release and the recapture location. The rectangle denotes 5% percentile of fish that have spent less than 50 days at liberty, demonstrating that at least some fish are able to travel long distances in relatively short time.

5.4 Discussion

The success of restocking programs requires not only releasing fish but also to improve the knowledge of the after-release life history and ecology of the target species

(Masuda & Tsukamoto, 1998). One of the key traits affecting success is dispersion capability, thus the movement patterns of released hatchery-reared meagres have been analyzed using acoustic telemetry, data storage tags and mark recapture methods, as a part of the restocking pilot experiments carried out in the Balearic Islands.

The results obtained from the data storage tags showed evidence of diel differences in the range of depths occupied by the released meagres. This range was wider and more variable at night than during the day. The maximum depth reached at night-time was 64.3 m but some records at depth lower than 1 m were also obtained. During the day, the fish usually remained at depth lower than 20 m but, more interestingly, it seemed nearly constant. Previous studies on distribution of meagre juveniles were based on commercial landing (Quéro & Vayne, 1987) or on experimental fishing (Catalán *et al.*, 2006b). Meagre juveniles were located between 20 and 40 m depth in the coastal waters near of Gironde (Quéro & Vayne, 1987) or between 5 and 10 m depth in open waters nearby the Guadalquivir mouth and along the coast throughout the year (Catalán *et al.*, 2006b).

The night-time changes in depth may be attributed to vertical movements in the water column, to horizontal displacements near the bottom, or even by a combination of both types of displacement. Nevertheless, the data provided by acoustic telemetry and mark recapture support that, at least in part, horizontal displacements are very important. However, direct support that this species moves all the time close to the bottom is still lacking, but it is a plausible working hypothesis.

During the day, the depth range was nearly constant which strongly support fish were not moving. It is probable that fish spend the day-time attached to the bottom, and hidden in order to avoid predation because to remain static near the sea surface or at mid-water would be too risky. This behavior is consistent with the information given by local spear fishermen during the interviews performed when they recaptured some meagres. Spear fishers stated that meagres remain immobile and hidden under rocks or whitin *Posidonia oceanica* meadows during the day. Instead, anglers stated that they catch meagres during sunset, sunrise or at night time. This reduced day activity was also observed in mulloway *Argyrosomus japonicus* (Taylor *et al.*, 2006), with both large and small fish remaining hidden within holes. Nocturnal and crepuscular predation is common among other members of Sciaenidae including *Plagioscion squamosissimus*

(Hahn *et al.*, 1999), *Argyrosomus japonicus* (Hecht & Mperdempes, 2001) and *Larimus breviceps* (Soares & Vazzoler, 2001). This photoperiod-dependent activity may relate to both increased foraging efficiency and minimisation of predation risk (Hodgson, 1972).

It was also remarkable that during the first day after release, this diel patter is not followed and fish exhibited higher variability in the depth range during day-time. This abnormal behaviour could be due to fish are generally stressed after transport from the hatchery to the release site. When stress is not severe enough to cause death directly, its indirect effects may still lead some behaviour-related mortality, for example by increasing predation likelihood (Olla *et al.*, 1998). Post-release predation is expected to be larger during the adaptation process because abnormal behaviour has been widely reported in cultured fish just after release (Kellison *et al.*, 2000; Stunz & Minello, 2001; Shimizu *et al.*, 2008), such as a lack of burying (Ellis *et al.*, 1997; Fairchild & Howell, 2004), longer times of off-bottom feeding (Furuta, 1996), and being at exposed sites, at mid-stream or far from refuges (Weiss & Kummer, 1999; Teixeira *et al.*, 2006). Release of fish during a period when they usually are inactive may lead to confusion, inability to seek appropriate habitats, conspicuousness and, subsequently, it increases predation risk (Olla *et al.*, 1998). However, the expected behavior of predation avoid (i.e., low movement during the day) was observed from the second day after release (Fig. 5.6), so a quick adaptation to the wild habitat was observed for this specific trait.

The maximum swimming speed observed in released meagres was 3.0 km h^{-1}(0.83 m s^{-1}). Meagres are expected to be morphologically specialized for acceleration but not for sustained cruising, as shown by the aspect ratio of the caudal fin (Pauly, 1989). A pelagic fish as yellowfin tuna (*Thunnus albacares*) showed a maximum mean swimming speed of 1.9 m s^{-1}, although some peaks round 3.0 m s^{-1} were observed (Marsac & Cayré, 1998). However, the crude data obtained from acoustic telemetry (remember that they are receiver positions rather than fish positions) does not allow accurate estimation of the horizontal movements at small scale (Marsac & Cayré, 1998). The detection probability is distance dependent at uses to be sigmoid-shaped (Gjelland & Hedger, 2013). Therefore fish can be at any point within a circular range around the receiver. Nevertheless, possible bias related with this uncertainty has been minimized here after removing the consecutive detections coming from closely located receiver. Therefore, the resulting estimate should be considered conservative in the sense that it reduces the chance of overestimation. Besides, this estimation was based on the shortest possible route between two points, so reducing overestimation risk too. Overall, even that some uncertainty remains on the swimming speed estimate for meagre, it should be concluded that swimming capability of meagre should be considered high in comparison with other coastal fish. Better estimation of swimming activity would be obtained using acceleration data storage tags (Furukawa *et al.*, 2011) or by re-analyzing the data with a movement model that explicitly takes into account the observational error related with acoustic data.

Concerning the type of movement, the huge difference (Fig. 5.8) between maximum speed at short scale (acoustic tracking; 3.0 km h^{-1}) and at large scale (mark-recapture, 0.54 km k^{-1}) strongly supports that fish should move speedy but tortuously, thus reinforcing that meagre could display a random walk type of movement, which is discussed below.

On the other hand, mark recapture data demonstrated a great dispersion capability. Released fish are able to travel long distances from the release site in few days. Fast movement from the release location to other areas with most suitable environmental conditions has been reported in other cases (Mitamura *et al.*, 2005). Although, in the case of released meagres, this behavior searching more suitable habitats is nearly implausible because some fish may remain near of (or come back to) the release site for a long time. Optimal release strategies, specifically size-at-release, release habitat and microhabitat, release season, acclimation, and density dependence, affect survival of released fish and need to be evaluated prior to large scale tests of restocking through pilot release experiments (Tsukamoto *et al.*, 1989; Willis *et al.*, 1995; Leber & Blankenship, 2011). The importance of the release habitat could be related with flow rates, habitat quality, prey, predator and competitor abundance (Leber *et al.*, 1996; Cowx, 1998; Jokikokko, 1999). Even, the release of fish in marine protected areas could improve their survival and the success of the restocking program (Purcell & Kirby, 2006). However, the high dispersion capability of the released meagres observed in this study prove that release habitat and release site are not relevant. Contrasting, the time of the day may be highly relevant because to release fish at night time, when they are more active, would reduce predation risk and thus enhance fish survival.

After combining the data obtained from the three methods (data storage tags, acoustic telemetry and mark recapture method), we propose a working hypothesis (a conceptual model) for the movement of released hatchery-reared meagres in the wild: 1) Fish remain static at the bottom during day-time. 2) Resting sites change between consecutive days (see below) but they are always close to the coast line (low depth). 3) After sunset, fish follow a random-walk type of movement or any other movement type implying tortuous trajectories. 4) During night-time fish probably move near the bottom, but some vertical movements on the water columns cannot be discarded. 5) Swimming speed at night time should be high. As a result, during night-time fish may reach points located relatively far from the coast and relatively deep. 6) Near sunrise, fish come back to the coast line, looking for a new resting site for day-time. 7) Successive cycles of day-nigth allows long-term dispersal around the island. Note that returning to the same resting site every day is incompatible with dispersing hundreds of kilometres in a relatively short time span. Note also that this conceptual model predicts that moving out of Mallorca Island is improbable because fish seem to rest at low depth during day-time. Nevertheless, this low-depth resting behaviour may change after some time at liberty or when fish are older and experience spawning migrations (González-Quirós *et al.*, 2011).

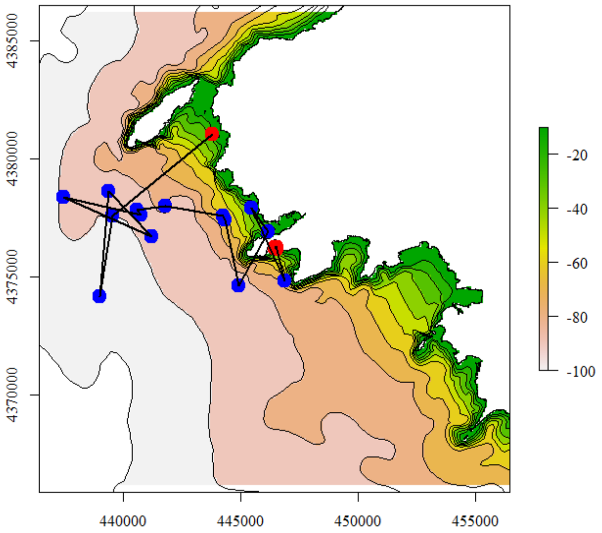


Fig. 5.11.- Movement pattern of one simulated fish during one day. The red points represent the fish position during the day and the blue points represent the position at night.

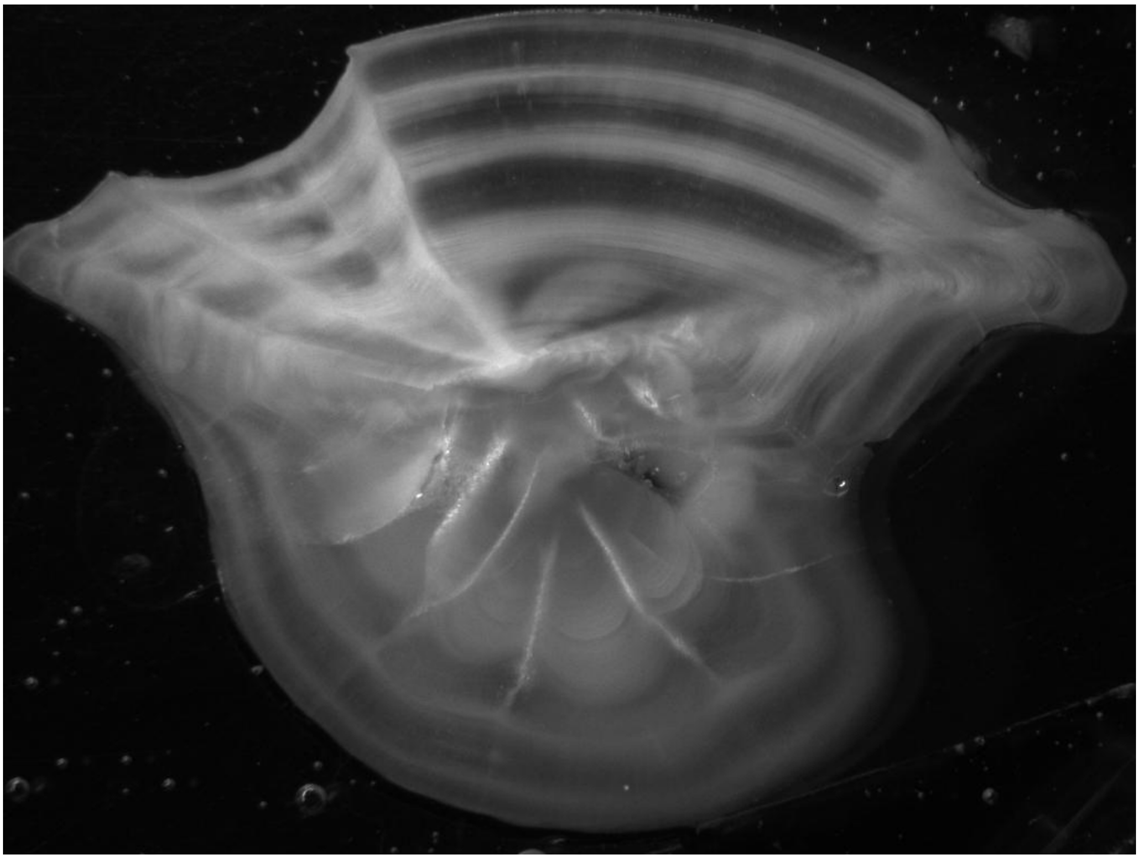
This conceptual model has been exemplified with a simulated trajectory starting at day-time of some day and finishing at day-time of the next day (Fig. 5.11). Each point represents a one-hour time step. During day-time (red points), the fish remain stationary in a shallow area. At night (blue points), the fish present a random walk movement, which results in reaching sites at highly variable depth range and with relatively large and fast displacements. Obviously, more research must be done for demonstrating the specific movement type displayed by meagre, and for obtaining reliable estimates of the parameters of this movement. In addition, more data are needed for confirming that meagre move close to bottom or when (at what age) fish start to experience spawning migrations. Nevertheless, this model explains two striking characteristics of meagre movement that may have a great impact on restocking success: 1) fish speed is surprisingly high and 2) dispersion capability is surprisingly large.

Chapter 6. Feeding adaptation of released hatchery-reared meagres into the wild

Released hatchery-reared juvenile meagre. (Photo: Enrique Massutí)

In: Gil, M.M., Palmer, M., Grau, A., Deudero, S., Alconchel, J.I., Catalán, I.A. (Accepted). Adapting to the wild: the cases of aquaculture-produced and released meagres. Journal of Fish Biology.

Chapter 7. Growth of released hatchery-reared meagres



Transverse section of meagre otolith.

In: Gil, M.M., Palmer, M., Grau, A, Pérez, S. (Submitted). The growth of hatchery-reared juvenile meagre Argyrosomus regius released in the Balearic Islands coastal region. Journal of Experimental Marine Biology and Ecology.

Growth of released hatchery-reared meagres

Abstract

The success of restocking depends on the capacity of phenotypes that are already adapted to captivity to readapt to the natural environment. Changes in growth rate after release can be monitored to determine whether released fish are adapting well to the natural environment or will fail to adjust to wild conditions. Well-adapted fish should at least grow at a rate similar to that observed prior to release. Nevertheless, it is not known whether individual fish experience a shift in growth rate. Alternatively, the fish showing long-term survival could be those that were already larger before release. This question is relevant for the maximization of stocking success because certain phenotypes (those with a better probability of readapting) could be selected for release. This Chapter compared the somatic growth of released and recaptured meagres, *Argyrosomus regius*, with control (captive) meagres belonging to the same cohort. Recaptures that had spent less than 3 months at liberty showed the same length-at-age as the control fish, but the length-at-age of most of the recaptured fish that had spent more than 3 months at liberty was greater than expected. This difference resulted from the combined effect of differential survival and increased growth. The otolith radius of the growth mark corresponding to the first year of life (i.e., when all fish were still in captivity) was significantly greater for fish that had spent more than 3 months at liberty, indicating that these meagres were larger and had a higher growth rate when they were released. Moreover, the analysis of daily otolith growth before and after release showed that most of the recaptured meagres that had spent less than 3 months at liberty grew an equal or lesser amount in the wild than before release. In contrast, most of the recaptures that had spent more than 3 months at liberty showed a higher growth rate after release.

7.1 Introduction

A. regius is one of the world's largest sciaenid fish, reaching more than 180-200 cm in total length and 50 kg in weight (Quéro & Vayne, 1987; Quéméner, 2002). It is a fast-growing species, especially during the first 5 years of life (González-Quirós *et al.*, 2011), and its growth shows wide variation. The reported asymptotic length at infinity

(L_∞) ranges from a total length of 171.9 cm (Gulf of Cádiz; González-Quirós *et al.*, 2011) to 210 cm (Mauritanian coast; Tixerant, 1974). Despite its rapid growth and high fecundity (Chapter 8), meagre is considered a highly vulnerable species.

Monitoring any change in growth rate after release may serve to indicate whether released fish are adapting to the natural environment or are failing in the feralization process (i.e., the capacity of phenotypes already adapted to captitivity to readapt to the natural environment; Lorenzen *et al.*, 2012). Juveniles must learn to capture live prey in sufficient numbers to sustain growth. However, many fish fail to master this task (Ersbak & Haase, 1983; Ellis *et al.*, 2002). A period of growth stagnation has been observed immediately after the release of hatchery-reared fish (Tomiyama *et al.*, 2011). In other cases, however, the released fish appear to follow the growth pattern of wild fish from the same age, indicating that adaptation is rapid (Paulsen & Støttrup, 2004).

Growth in fish has been shown to exhibit phenotypic plasticity in response to environmental and anthropogenic (typically, fishing) effects (Sinclair *et al.*, 2002; Helser & Lai, 2004; Alós *et al.*, 2010a; Alós *et al.*, 2010b). The two most important environmental factors affecting fish growth are food level and water temperature (Weatherley & Gill, 1987). Moreover, the different growth rates observed in the same cohort may involve phenotypic selection, as faster-growing individuals may have a higher or lower probability of survival (Takahashi *et al.*, 2012). The survival advantage of the larger or more rapidly growing members of a cohort is due to an improved resistance to starvation, decreased vulnerability to predators and better tolerance of environmental extremes (Sogard, 1997; Takahashi *et al.*, 2012). However, these fish may also be more vulnerable to fishing mortality (Swain *et al.*, 2007; Brunel *et al.*, 2013).

Body length (i.e., length-at-age) has been used to assess the effects of environmental variables on growth rate (Brunel *et al.*, 2013). Another method for analyzing the growth of fish is to use the growth marks produced in the otoliths (May & Jenkins, 1992; Suthers & Sundby, 1993; Suthers, 1996; Barber & Jenkins, 2001). This latter method assumes that otolith growth and somatic growth are well correlated. Validating the periodicity of formation of any growth mark is a mandatory procedure before applying otolith microstructure analysis. The daily periodicity of putative daily microstructures has been demonstrated for meagre over a wide age range (from 2 months to 2 years old;

Chapter 4). In certain species, in addition to species-specific ontogenetic changes, other factors may affect the width of the daily growth marks in the otolith (McCormick & Molony, 1992; Molony & Sheaves, 1998). For example, the feed regime and starvation may have substantial effects on increment widths in juvenile fish (Campana, 1983; Rice *et al.*, 1985; McCormick & Molony, 1992; Molony & Sheaves, 1998).

The objective of this Chapter was to evaluate the adaptation of released meagres by analyzing the growth pattern of released fish. Specifically, the somatic and otolith growth of released and control (i.e., still-captive) meagre was compared, and the observed differences were attributed to differential survival or to increased growth. The working hypothesis to be tested is that although fish may even experience a decrease in growth immediately after release, the growth rate after an adaptation period may be even more rapid than the growth rate of the same fish in captivity. In addition, the better-adapted fish would be expected to show improved survival. Therefore, this study will examine 3 types of evidence. Specifically, recaptured fish that had spent different amounts of time at liberty were analyzed in terms of 1) size-at-age, 2) estimated size when they were 1 year old and 3) daily growth rate before and after release.

7.2 Materials and Methods

7.2.1 Sample collection

7.2.1.1 Recaptured meagres

All the meagres returned to the lab (see Appendix Table I.4) were stored at -20 ºC. After defrosting, these fish were measured (total length and weight), and the otoliths were dissected for subsequent preparation.

7.2.1.2 Control meagres

The control group was composed of 1594 specimens of meagre born in 2007 and reared with the same methodology as that used for the released fish. These specimens remained under controlled conditions in sea cages (5.5 m diameter) at low density and were fed with commercial feed pellets (Skretting®, Burgos, Spain; Pastor & Grau, 2013). The basic sampling involved measuring the length and weight of control fish at known ages (from 0.7 to 4 years old). The sampling dates and sample sizes are detailed in Table 7.1. Note that the released fish were also considered to represent control fish before they were released. The rest of the control fish were part of the samples used in Chapter 8.

Sampling data	Number of individuals	Mean ± S.D. LT (cm)	Treatment
05/02/2008	15	22.8 ± 2.7	Sacrified
13/05/2008	15	24.5 ± 2.4	Sacrified
23/10/2008	20	32.3 ± 1.4	Sacrified
24/02/2009	1272	34.3 ± 3.4	Released
07/05/2009	25	36.9 ± 2.1	Sacrified
04/01/2011	8	64.8 ± 4.3	Sacrified
06/04/2011	10	64.4 ± 3.3	Sacrified
19/05/2011	10	67.9 ± 2.7	Sacrified
17/06/2011	10	66.8 ± 3.2	Sacrified

Table 7.1.- Sampled control meagre specimens born in 2007 (birth data: 06/05/2007).

7.2.2 Otolith preparation and observation of daily and annual marks

The left *sagitta* otoliths of 21 recaptured meagres that were released on February 2009 were cut transversely using protocol detailed in the Chapter 4.

Transverse thin sections were analyzed using a DMRA2 Leica microscope at x400. Four digital calibrated images were taken along a predefined radius using a Leica camera. The use of areas shared between images allowed the images to be combined into a single image. Previous pilot analyses had demonstrated that combining 4 images at the magnification scale used (or 400-500 µm) ensures that an area from the otolith edge to a date clearly prior to the release date would be covered. The counting and measuring of all DGMs along a predefined radius was performed as in Chapter 4 (Fig. 7.1). Two reading sessions were completed for each otolith. The otolith radius for reading was manually selected and its location was nearly, but not exactly, the same for the two sessions. All of the reading sessions were completed by the same reader, who was blind to the identity of the fish. The order in which the otoliths were read was random, and an interval of several days elapsed between the two reading sessions for the same otolith.

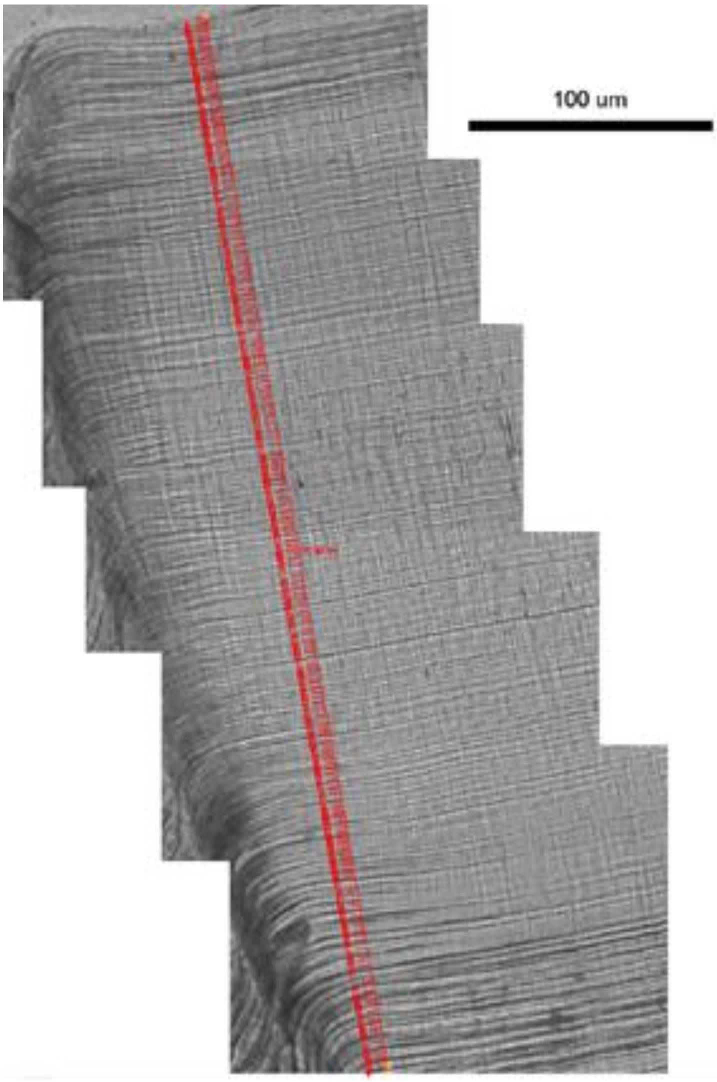


Fig. 7.1.- Reading daily increments on the selected radius of an A. regius otolith transverse section. The red lines indicate the observed location of each daily increment for one specific reading session.

To examine the annual growth marks, 102 thin transverse sections from 35 recaptured meagres that had experienced contrasting (i.e., short or long) periods of release (Appendix Table I.4), 8 control meagres from the same cohort and 59 control-reared meagres used in Chapter 4 were processed to determine the otolith radius (R_O, mm; distance from the core to the edge following the *sulcus acusticus*). The radius was measured by applying ImageJ software to calibrated images of the sections. This data set was used to test the relationship between otolith size and fish body size. Body size (L_T) ranged from 12.7 to 79.1 cm. In addition, the radius from the core to each annual growth mark on the same radius was measured for the 35 recaptured meagres. The interpretation of annual growth marks followed the protocol proposed by Prista *et al.* (2009).

7.2.3 Data analysis 1: Size-at-age

The length-at-age relation of the control meagres was adjusted to the growth model proposed by Somers (1988), which is a version of the conventional Von Bertalanffy (VB) growth model that incorporates seasonal growth oscillations (García-Berthou *et al.*, 2012). This model was fully described in Chapter 3, and also used in Chapter 4. In this case, L_0 was the mean length at release and t_0 was the age at release (Table 7.1).

As in the Chapter 3, L_∞ was assumed to be 171.9 cm (González-Quirós *et al.*, 2011) and the other parameters (C, K and t_S) were estimated using a Bayesian approach. The values of the parameters C, K and t_S obtained for meagre fed with the used diet (A; commercial meagre pellets; Chapter 3), were assumed as priors. As in Chapter 3 the model was also implemented with the library *R2jags* of the R-package.

The observed length-at-age of the recaptured meagres was compared with the expected length-at-age estimated from control meagres of the same age to determine whether the recaptures followed the same pattern as the control fish. In addition, we tested for a possible difference in the growth pattern between the recaptures that had spent less than 3 months at liberty and those that had spent more than 3 months. This threshold was selected because, as it was shown in Chapter 6), released meagres appear to return to good biological condition and to shift to a more piscivorous diet after spending approximately 3 months at liberty. Visual inspection (see Results) suggests that the meagres recaptured after more than 3 months have a different growth pattern than control meagres and recaptures that had spent less than 3 months at liberty. Therefore, two additional analyses were completed to test whether the observed differences were due to (1) the differential survival of individuals with different growth rates or (2) increased growth in the natural environment.

7.2.4 Data analysis 2: Differential survival

Possible differences in survival depending on the growth rate variability of fish from the same cohort were examined by analyzing the size of the recaptures at the first year of age, i.e., when all of them were in the same lab-controlled rearing conditions. Specifically, we tested whether the specimens with a higher growth rate (or, equivalently, fish with a larger size-at-age because all fish were born and released on the same dates) had a higher probability of survival in the natural environment. This objective was achieved by analyzing the otolith radius of the 32 returned meagres (those from the 2007 cohort; 1 to 961 days at liberty) corresponding to the age of one year. The otolith size at one year was compared between the specimens that had spent less than or more than 3 months at liberty. Note that it is possible to infer the body length of a fish at younger ages from the width of the annual increments recorded in the otoliths (Pilling *et al.*, 2002) only if there is a strong relationship between otolith length and fish body length (as verified for *A. regius*, see below). Significant differences between groups in the otolith size corresponding to an age of one year were tested using a one-way ANOVA. Homogeneity of variances and normality of residuals were examined using a Bartlett test and a Shapiro test, respectively.

7.2.5 Data analysis 3: Comparing growth before and after release

Possible growth changes experienced by a fish between the period before release and the period after release were analyzed for a subsample of the recaptured fish. Daily growth marks (DGMs) of 9 recaptured meagres that spent different times at liberty were analyzed. The log-transformed width of the daily increments (I_W) was assumed to be linearly related to age. Note that this assumption is implicit in the conventional VB model (Palmer *et al.*, submitted). The presence of a slope change between the periods before and after the known release date would indicate a growth change. However, as the observed pattern may be masked by the effects of environmental covariables on meagre growth (see above), water temperature was added to the model. Therefore, the model fitted to each fish was as follows:

$$\log(Iw_i) = \beta_0 + \beta_1 Age_i BEFORE_i + \beta_1 (Age_R - DAL) AFTER_i + \\ \beta_2 (Age_i - (Age_R - DAL)) AFTER_i + \beta_3 Age_i Temp_i$$

where i ranges from 1 to the N detected DGMs, Age_i is the number of days since the first measured daily increment, Age_R is the number of days from the first measured daily increment to the recapture (i.e., the edge of the otolith) and DAL is the number of days that the fish has been at liberty. $Temp_i$ is the sea surface temperature. $BEFORE$ is an auxiliary variable taking the value 1 if Age_i is less than the date of release (the fish is still in captivity) and zero otherwise. $AFTER$ is defined in the opposite manner. Based on this parameterization, the growth rate before release is estimated by the first two terms only (β_0 and β_1). After release, the model includes not only the growth before release (β_0 and β_1) but also the additional growth after release (β_2).

The identification and measurement of the daily increment involve several sources of uncertainty linked to the preparation of otoliths, the observation of structures a few microns in size (e.g., Zhang *et al.*, 1991; Fey *et al.*, 2005) and the interpretation of these structures (i.e., identification, enumeration and measurement of DGMs; Morales-Nin & Panfili, 2002). A failure to identify one or more daily increments (*skipping*) implies the overestimation of the average growth rate and the underestimation of age. Therefore, potentially skipped DGMs were detected and estimated using the method developed by Palmer *et al.* (submitted): two reading sessions are combined to model growth and age, incorporating the probability that a DGM was skipped. The detection probability was estimated from the cases for which a DGM was detected in only one of the two reading sessions of the same otolith. The method is based on assigning two or more days if an increment is larger than expected. This method allowed us to estimate the values of the parameters β_0, β_1, β_2, β_3 and Age_R and to detect differences between the growth rate before and after release (if $\beta_1 \neq \beta_2$). The model was implemented with the library *R2jags* (http://cran.r-project.org/web/packages/R2jags/R2jags.pdf) of the R package (at http://www.r-project.org/), which uses the samplers implemented in JAGS (http://mcmc-jags.sourceforge.net/).

7.3 Results

7.3.1 Somatic growth of control and recaptured meagres

The data from the control meagres were successfully fitted by the proposed model using the known values for L_0 (34.3 cm) and t_0 (660 days). The length-at-age data for the recaptured meagres that had spent less and more than 3 months at liberty were added to the growth curve fitted to the control fish (Fig. 7.2). Almost all of the recaptures that had spent less than 3 months at liberty had a length within the 95% Bayesian credibility interval of the expected length-at-age of fish of the same age but reared in captivity (i.e., control fish). In contrast, the length-at-age of most of the recaptured fish that had spent more than 3 months at liberty were outside (larger than) the corresponding credibility intervals (Fig. 7.2).

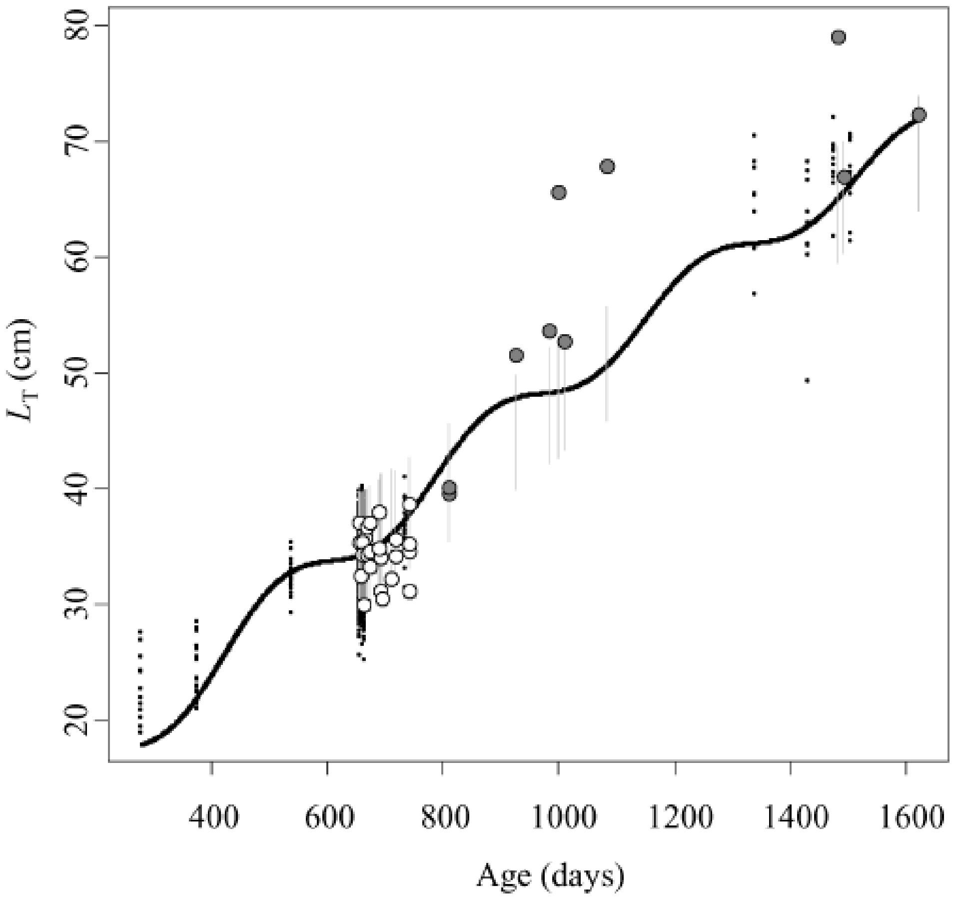


Fig. 7.2.- Growth model with seasonal oscillation for the control *A. regius*. The length-at-age data of recaptured *A. regius* that had spent less (white) and more (grey) than 3 months at liberty has been added to the control growth model. The vertical grey lines show the expected length (including confidence intervals between 25% and 75%) for the recaptured specimens.

The residuals (observed length minus expected length for a control fish of the same age; Fig. 7.3) for the recaptured meagres that had spent more than 3 months at liberty were significantly greater than those for the recaptured fish that had spent less than 3 months at liberty ($F_{1,\ 34} = 15.2$; $P < 0.05$). Therefore, the recaptured meagres that had spent more than 3 months at liberty were larger than expected. This pattern may be the result of differential survival of the largest specimens, of increased growth in the wild, or both.

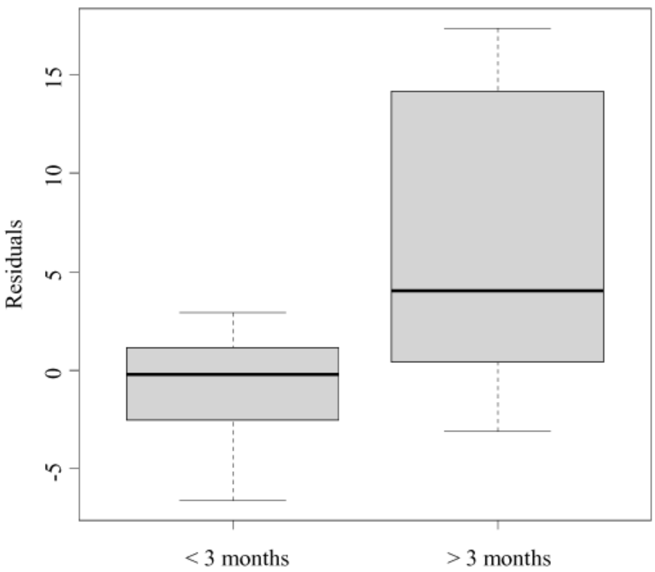


Fig. 7.3.- Boxplot of residuals from the estimates obtained from the fit of the recapture data to the control growth model and comparison of the recaptures that had spent less and more than 3 months at liberty. A line within the box marks the median values, the boxes the 25 and 75% percentiles and the bars the minimum and maximum non-outlier values.

7.3.2 Differential survival

Fish body length and otolith radius followed a positive linear relationship (Fig. 7.4; r^2 = 0.94), suggesting that otolith length can be used as a reliable proxy for somatic length.

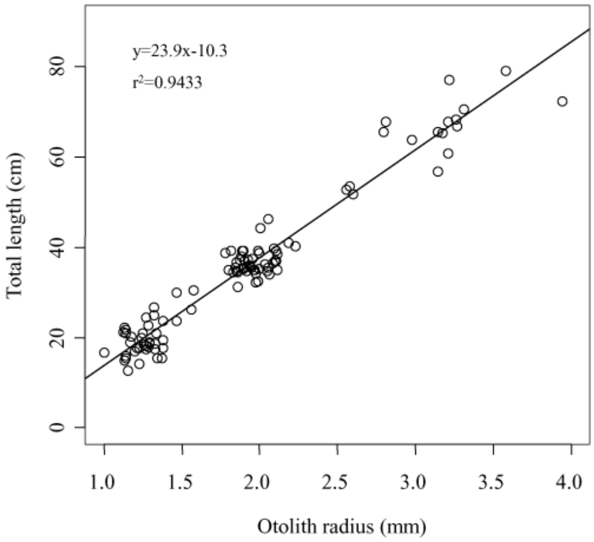


Fig. 7.4.- Linear regression between the fish body total length and otolith radii of *A. regius*.

For this reason, the otolith radius at the first year was used to compare the estimated body length of recaptured fish that had spent less and more than 3 months at liberty. This comparison showed that the recaptures that had spent more than 3 months at liberty showed an otolith radius at the first year that was significantly larger ($F_{1,30} = 6.17$; $P < 0.05$) than that for the recaptures that had spent less than 3 months at liberty (Fig. 7.5). Therefore, the meagres recaptured a long time subsequent to release were already significantly larger at the release date than the meagres recaptured a short time after release.

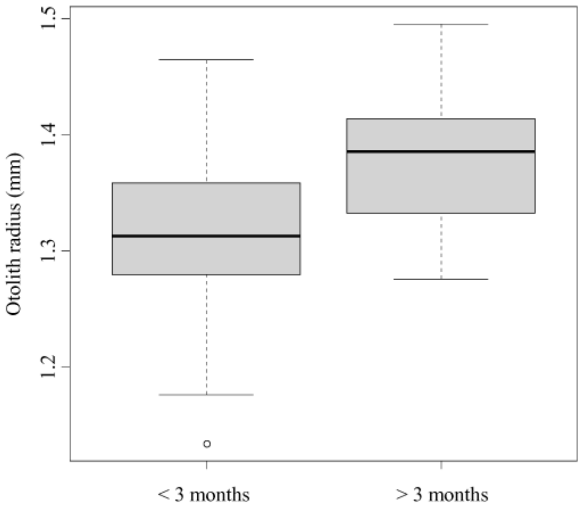


Fig. 7.5.- Boxplot of otolith radii at the first year for *A. regius* recaptures, comparing fish that had spent more and less than 3 months at liberty.

A Bartlett test and a Shapiro test showed that the data examined had homogeneous variances ($P > 0.05$) and normally distributed residuals ($P > 0.05$).

7.3.3 Increased growth after release

The method used in this study allows the skipped DGMs to be estimated. Therefore, the release date can be located from the readings of the otolith radius, and the growth of the otolith before and after release can be compared (Fig. 7.6). Note that the pattern obtained from this analysis is observable in addition to the effects of temperature on growth because temperature was added to the model as a covariable. The number of skipped DGMs identified suggests that to ignore skipping would produce an overestimate of the averaged growth rate and an underestimate of the age. A few probable sub-daily growth marks were also identified in several fish, but the small number of these marks indicates that it is implausible that they affected the growth rate estimates.

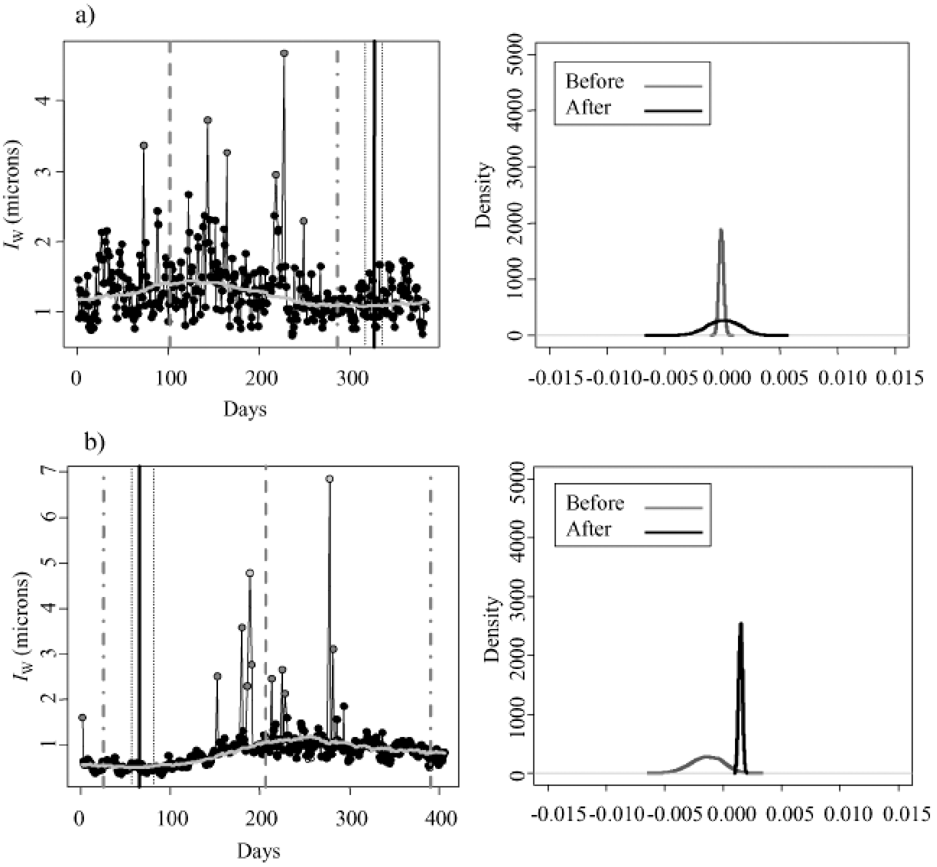


Fig. 7.6.- Estimated daily growth increments (left) and growth rate before and after release (right) for a) meagre R13, which had spent less than 3 months at liberty and b) meagre R33, which had spent more than 3 months at liberty. In the left-hand graph, the black points represent the true identified daily increments, the grey points the skipping increments (dark grey = 1 skip and light grey = 2 skips). The continuous black vertical line represents the estimated location of the release date. The discontinuous grey vertical lines show the maximum and minimum temperature.

This method failed to converge for meagres which had spent a few days at liberty because the high among-day variability in growth prevents the reliable estimation of the growth rate after release. In addition, a result of the complex process of otolith preparation was that DGMs were not interpretable for several other otoliths. Therefore, only 9 meagre otoliths could be properly analyzed. The range of days at liberty for these 9 otoliths was from 35 to 422 days. The difference between the estimated growth rate before and after the release was variable, suggesting that certain meagres grew more in the wild but that the growth of others was less than or similar to the amount of growth in captivity (Table 7.2).

ID	DAL	Differential growth rate
R4	35	-0.006
R13	56	0.000
R22	81	0.000
R24	81	0.008
R25	81	0.000
R27	156	-0.008
R28	156	0.003
R33	340	0.003
R44	422	0.008

Table 7.2.- Days spent at liberty (DAL) and differential otolith growth rate (μm day-1) before and after release for the 9 analyzed meagre otoliths.

Interestingly, most of the meagres that had spent less than 3 months at liberty showed a growth rate in the wild similar to or lower than the growth rate before release (Table 7.3). Instead, most of the meagres that had spent more than 3 months at liberty showed a higher growth rate in the wild than in the previous reared condition, and only one fish had a lower growth rate. However, the low number of otoliths for which reliable growth rates have been obtained prevents a proper statistical comparison of the general trends found for all fish.

	< 3 months	> 3 months
Before ≥ After	4	1
Before < After	1	3

Table 7.3.- Number of *A. regius* otoliths that showed a growth rate higher or lower/equal in the wild relative to the previous reared condition for fish that had spent less or more than 3 months at liberty.

7.4 Discussion

A high growth rate in released fish may indicate that they have adapted well to wild conditions and have good prospects of survival. Satisfactory growth cannot be achieved if released fish are unable to obtain sufficient prey. For this reason, the estimation of the post-release growth performance of returned fish is an indirect way to evaluate the effectiveness of restocking (Wada *et al.*, 2010; Tomiyama *et al.*, 2011).

However, the length-at-age of returned fish has little value in itself as an indicator of the adaptation of fish to the wild. Control fish of the same age maintained in captivity furnish a good reference value for comparison. However, the growth of meagre appears to depend on not only age but also environmental factors. The growth model proposed by Somers (1988), which incorporates seasonal growth oscillations (García-Berthou *et al.*, 2012), was chosen to fit the length-at-age data on meagre specimens maintained in captive rearing conditions. Thereby, the strong influence of seasonal factors, such as temperature, light and food supply, on meagre growth was included in the model of growth (Chapter 3). The growth rate of meagre decreases substantially if the temperature is less than 16 ºC (Quéméner, 2002; El-Shebly *et al.*, 2007). This pattern of decreased growth at lower temperatures has also been observed in mulloway *Argyrosomus japonicus*, in which high growth has been reported at warmer temperatures (Taylor *et al.*, 2009). Therefore, the incorporation of seasonal oscillation in the growth model has allowed us to obtain more reliable estimates of length-at-age and to compare them with those currently observed for the returned fish.

The returned specimens showed that at least some meagres remained around the coastal areas of Mallorca Island for at least 2-3 years (Appendix Table I.3). The growth performance of returned fish is high. Depending on the cohort, fish can grow from a size of 43.2 cm at release to 77 cm after 1.8 years at liberty or from a size of 34.2 cm at release to 79.1 cm in 2.6 years. The length-at-age of the returned meagres was compared with that of the control fish, and clear differences between specimens that had spent less and more than 3 months at liberty were observed. The recaptured meagres that had spent less than 3 months at liberty presented lengths-at-age similar to or even lower than those expected for the control meagres. This result is consistent with the results reported in Chapter 6, based on the condition index, stable isotopes and stomach contents, which demonstrate that released meagres need several months (approximately 3) to adapt to wild conditions. This adaptation period appears to be variable and species-specific, but the same growth stagnation has been observed in Japanese flounder (*Paralichthys olivaceus*) for several days after release (Tomiyama *et al.*, 2011). In contrast, comparisons of growth rates between wild and reared turbot (*Psetta maxima*) showed similar growth patterns, indicating that the reared fish were able to adapt rapidly to natural conditions (Paulsen & Støttrup, 2004).

Most of the returned meagres that had spent more than 3 months at liberty showed a higher growth rate than that expected from control fish of the same age. This result suggests that fish may increase their growth in the wild. The use of methods such as otolith microstructure can reveal changes in growth rate associated with the process of adaptation to the natural environment. The otolith represents an integration of the entire growth history of a fish (Burke *et al.*, 1993). Several studies have demonstrated that it is possible to estimate the growth rate by measuring increment widths (Campana & Neilson, 1985; Hovenkamp & Witte, 1991; Stunz *et al.*, 2002). The two key assumptions for using the growth marks on the otolith to estimate somatic growth are that 1) the distance between increments must be proportional to the somatic growth occurring between the corresponding dates and 2) the periodicity at which marks are deposited must be verified (Campana & Neilson, 1985). These assumptions have been verified for both annual (Prista *et al.*, 2009) and daily growth marks (Chapter 4). Food level directly influences otolith growth (Jenkins *et al.*, 1993; Paperno *et al.*, 1997; Barber & Jenkins, 2001). For example, significantly higher otolith growth has been observed in fish fed a high food ration at 18 ºC and 12 ºC (Barber & Jenkins, 2001). However, daily increment deposition during starvation can be observed for a certain time (Campana, 1983) because body fat reserves may provide sufficient energy for otolith growth (Jobling, 1980; Marshall & Parker, 1982). This point is important because the low stomach contents and low condition index observed in meagre juveniles (Chapter 6) immediately following release may imply a relatively long starvation period during which the (otolith) growth rate is expected to be small but positive.

The comparison of the growth rate estimated from DGMs between the periods before and after release in recaptured meagres in the Balearic Islands showed consistent variations in growth. The growth of returned meagres that had spent less than 3 months at liberty was similar to or even slower than their previous growth in captivity. This finding is consistent with the view that the fish were still in an adaptation period. Interestingly, however, most of the recaptured meagres that had spent more than 3 months at liberty and, therefore, should be considered well adapted to wild feeding showed an increased growth rate after release.

We propose that this shift in growth rate may be related to a corresponding shift from a diet based on invertebrates to a more piscivorous diet. This statement is supported by not only the diet shift observed in returned fish (Chapter 6) but also the finding that a semi-moist fish-based diet produces a better growth rate than diets based on any commercial pellets (Chapter 3). Similarly, wild 110 g meagre juveniles reared in sea cages reached 1,850 g in only 8 months if fed with fresh fish (Pastor *et al.*, 2002). Therefore, although it is probable that more energy must be invested in catching fish in the wild, the high efficiency of meagre when consuming fish suggests that well-adapted fish (fish recaptured after more than 3 months at liberty) grew better than the control meagres maintained under the rearing conditions and fed with commercial pellets. However, it should be emphasized that reliable estimates of the growth rate before and after release have been obtained for a relatively small number of fish. For this reason, the sample size should be increased.

In another species, *P. olivaceus,* the increase in growth observed after a release-related period of growth stagnation (Tomiyama *et al.*, 2011) was attributed to compensatory growth (Ali *et al.*, 2003). Compensatory growth is a phase of accelerated growth occurring when favorable conditions are restored after a period of growth depression. The original growth trajectory is restored, and fish may achieve the same length-at-age as conspecifics experiencing favorable conditions at any time (Ali *et al.*, 2003).

In addition to the increased growth rate of well-adapted fish (i.e., those that had spent more than 3 months at liberty), the otolith radius when fish were one year old (i.e., when all fish were still being reared in captivity) showed that those well-adapted fish were also larger at the release date in comparison with fish that had spent less than 3 months at liberty. This result suggests that fish that are larger at the release date may adapt better to wild conditions, growing faster and surviving better than smaller fish. Therefore, fish length should have a major impact upon the recapture rates (and presumably survival) of released juveniles (Leber, 1995) due to the greater impact of predation on smaller organisms (Tsukamoto *et al.*, 1989; Ray *et al.*, 1994). A higher survival rate in more rapidly growing fish (i.e., high-quality fish) has also been observed in *P. olivaceus* (Tomiyama *et al.*, 2011) and *A. japonicus* (Taylor *et al.*, 2009). However, this pattern may be reversed or at least modulated over the long term if fishing mortality is the principal source of mortality because fisheries-induced evolution tends to select smaller-sized fish (Brunel *et al.*, 2013). The focus of the current study was only the short and critical period during which released fish must adapt to dramatically different conditions or die.

In conclusion, a higher-than-expected length-at-age was observed in released meagres that had spent several months at liberty, but this growth pattern could be observed only for survivors, i.e., those fish that appeared to have successfully adapted to wild conditions and survived. These long-time survivors were the largest members of the cohort. Their large size implies not only a lower vulnerability to predation but also a greater competence in obtaining food and, therefore, a greater adaptability to wild conditions. The relationships between the fish quality, post-release growth and survival of released juveniles imply that stocking effectiveness could be improved by selecting specific phenotypes during juvenile production (Tomiyama *et al.*, 2011). Therefore, improvements in the production protocols and in the release strategies could enhance the adaptation of the released juveniles and increase their potential survival after release.

Chapter 8. Reproductive strategy and fecundity of meagre

Eggs collected from the hormonal induced meagre broodstock.

In: Gil, M.M., Grau, A., Basilone, G., Ferreri, R., Palmer, M. (2013). Reproductive strategy and fecundity of meagre Argyrosomus regius Asso, 1801 (Pisces: Sciaenidae): implications for restocking programs. Scientia Marina 77(1), 105-118.

Chapter 9. Survival of released meagres

Recaptured meagre in a fish market. (Photo: Oliver Navarro)

Survival of released meagres

Abstract

The final objective of any fish restocking program should be to recover the target stock in a way that it could be again sustainably exploitable. At a relatively short-term, one way to check the potential success of a restocking program is to ensure that at least some of the hatchery-reared and released fish were able to survive until sexual maturity, thus they were able to reproduce at the wild. The objective of this Chapter is to estimate the mortality rate of the released fish and, thus, to estimate how many of the *ca*. 10000 released meagres are expected to reach sexual maturity. Recaptured fish from the commercial and recreational fisheries have been used for this purpose. However, direct estimation of mortality rate from the ratio recaptured/released would naively overestimate mortality rate because of two problems: non-reporting (some tagged fish are captured but they are not reported) and tag-loosing (Chapter 4). An unbiased method, robust against these two problems, has been proposed based on analyzing the temporal distribution of recaptures instead of the recaptured/released ratio. As inferring mortality in such a way may be counter-intuitive, we first completed a number of computer-simulations demonstrating that this method is robust for a wide gradient of realistic values of mortality rate and reporting rate. In addition, this method is robust even when returned fish that have spent short time at liberty are excluded from the analyses. The latter is relevant because two main sources of mortality for hatchery-reared and released meagres have been hypothesized to significantly affect survival: 1) short-term natural mortality related with the adaptation process to the wild and 2) fishing mortality. When all the returned meagres were included, the estimated mortality rate (0.0169) was probably overestimated because of abnormal short-term mortality. When fish that have spent less than 90 days at liberty was excluded, estimated survival rate (0.0029) would be more realistic for long-term prediction. In the latter case, the number of fish that is expected to reach sexual maturity is more difficult to be estimated, but it should move between 5 and 25. Note that if fish could skip such a high mortality period (90 first days), the corresponding number of fish reaching sexual maturity would be 2326. Therefore, releasing strategy should be improved in two ways: 1) increasing the number of released fish and 2) reducing the short-term mortality rate.

9.1 Introduction

The proper approach when using restocking for stock enhancement involves undertaking pilot studies in order to provide the scientific-based knowledge for designing restocking strategies that maximize benefits on the target stock and minimize environmental impacts (Blankenship & Leber, 1995). Short-term effect of pilot trials has been reported for punctual (small and spatially localized) releases of highly depleted species. In these cases, releasing was closely followed by increased landings (Wada *et al.*, 2010). However, long-term success should be confirmed by a sustainable population dynamic, which in practice is a very challenging objective, especially for a long-lived species that reach sexual maturity late in life, as is the case of meagre. An alternative way for assessing the potential success of a restocking program is to estimate the survival capability of released hatchery-reared fish to reach sexual maturity in sufficient numbers to rebuild a population with a stable age structure and to ensure the next generations of broodstock (Ireland *et al.*, 2002).

Two main sources of mortality for hatchery-reared and released meagres have been hypothesized to significantly affect survival: 1) short-term natural mortality related with the adaptation process to the wild and 2) fishing mortality. Adaptation to natural habitats and natural food sources is critical for survival of hatchery-reared fish (Tomiyama *et al.*, 2011). Recent studies have revealed the poorly developed behavioral skills in released juveniles, such as feeding or burying ability and escape response from predators (Furuta, 1996; Ellis *et al.*, 1997; Kellison *et al.*, 2000). Post-release predation can be expected to have a relevant impact during the adaptation process because these behavioral anomalies (Furuta *et al.*, 1997). Although conditioning and training before release have been reported to mitigate such mortality (Sparrevohn & Støttrup, 2007), the key to better survival of released juveniles seems to be to optimize the release strategy, i.e. release at habitats, seasons, juvenile sizes and numbers of released fish that maximize survival (Wada *et al.*, 2010).

Therefore, the objective of this Chapter is to estimate the long-term survival rate of hatchery-produced and released meagres and, thus, properly evaluating a long-term survival from short-term abnormal mortality related with the adaptation to the wild. Meagre abundance has decreased alarmingly in Mediterranean waters (Quéro & Vayne, 1987; Sadovy & Cheung, 2003) at an extent that it is considered extinct in the Balearic

Islands (western Mediterranean), where it was a relatively frequent capture only a few decades ago (Riera *et al.*, 1997; Mayol *et al.*, 2000). Therefore, meagre was selected as a target species for a restocking program. The meagre restocking program released the first batch of hatchery-reared fish in 2008. Since then, a total of 13134 meagre juveniles have been released in the Balearic Islands.

However, to estimate the survival rate of this large amount of released fish is more challenging than expected. Capture-mark-recapture methods have been largely applied for estimating survival, population abundance and population dynamics (Cormack, 1964; Jolly, 1965; Seber, 1965). From these early contributions, an impressive development on theoretical, statistical and technical aspects has been done (Kery & Schaub, 2012). Nevertheless, most of the robust methods currently available are funded in disentangling capture probability and survival probability, which is usually done by accurate monitoring of few individuals for which a relatively long history of encounters (or recaptures) is precisely known (Kery & Schaub, 2012). Unfortunately, this sampling strategy is precluded for most marine organism because, in most of the cases, the only way to obtain information from a marked and released fish is when these fish were captured. Thus data typically consist in only-one recapture histories (i.e., single-tag recoveries).

Lincoln-Petersen's index (Petersen, 1896; Lincoln, 1930) and other related methods use the number originally tagged at the denominator. Therefore, the main limitation of this type of method when estimating mortality rates from single-tag recoveries is that tag non-reporting, tag-induced mortality, and tag shedding, can severely bias these estimates if these losses of tagged animals from recapture sample are not accounted for (McGarvey *et al.*, 2009).

Some methods have been developed to estimate the rate of tag non-reporting. These include offering a very high reward for some special ("golden") tags, which are assumed to be returned in any case (Pollock *et al.*, 2001; Cadigan & Brattey, 2003; Cadigan & Brattey, 2006), using on-board observers to monitor tag recoveries (Hearn *et al.*, 1999; Pollock *et al.*, 2002a; Eveson *et al.*, 2007), seeding portions of the catch with tags, and observing the fraction reported (Hampton, 1997; Hearn *et al.*, 2003), and by a combination of high reward tags and observers (Pollock *et al.*, 2002b).

Instead, Chapman (1961) proposed an estimator of total instantaneous mortality rate that uses only the mean of tag-recovery over time. More recently, this method has been proved to be unbiased for a constant average rate of tag non-reporting, a constant rate of tag losses and a constant rate of tag-induced mortality (McGarvey *et al.*, 2009).

This simple approach is funded in analyzing the temporal distribution of the number of returned fish in time, which is assumed to be exponential (McGarvey, 2009). Nevertheless, inferring mortality in such a way may be counter-intuitive. Therefore, we first completed a number of computer-simulations demonstrating that this method is robust for a wide gradient of realistic values of mortality rate and non-reporting rate for the meagre.

Then, the method was applied to the actually observed data for the released meagres. Nevertheless, independently of the method used for analyzing the data, the only source of information of the released fish is the fish captured by commercial and recreational fishermen. Therefore, fishermen need to be readily informed of the releasing events, thus to develop appropriate advertising campaigns at the media or using any other way to motivate the fishers is essential for maximizing the number of returned fish and thus obtaining more precise and accurate estimates of fishing mortality. In this Chapter, we also reported the method used for maximizing the number of returned fish.

9.2 Materials and Methods

9.2.1 Advertising campaign

An advertising program was designed and implemented to inform the commercial and recreational fishermen about the meagre restocking program development and the steps to take if a meagre was caught. The communication program consisted of i) television, radio and press appearances, ii) poster and leaflet distribution, and iii) personal interviews.

In several occasions, the scientists involved in the meagre restocking program have appeared in local media, such as television, radio or press, to inform the population about the objectives of the project. Furthermore, just before each release event, informative posters (Fig. 9.1a) were distributed to all the fishing shops, diving communities and professional fishermen's associations in Mallorca. Leaflets were mainly (but not only) distributed at Palma Bay, the area with greater influx of recreational and commercial fishermen. Finally, personal face-to-face interviews were also performed to obtain data on the penetration of the advertisement campaign among the fishers and to obtain additional data on non-reported captures of meagre. These interviews were also exploited for explaining the details of the restocking program to the fishers and to instruct them about what to do when recapturing a tagged meagre.

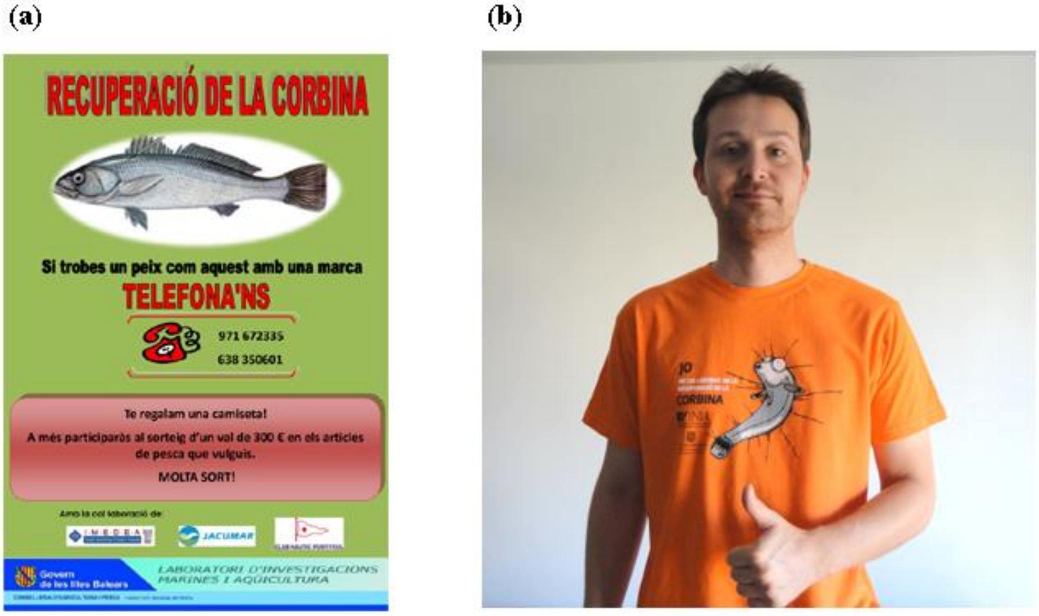


Fig. 9.1.- (a) Poster describing the meagre restocking program and instructions for rewards. (b) T-shirt gift for each recapture information.

When a tagged meagre was recaptured and the fishermen called us on the phone number printed on the T-bar tag, they provided information about the recapture date and location (as precisely as possible), approximate depth of fishing, type of gear and approximate length or weight. A reward was given to fishermen who collaborated reporting information of recaptured meagres. The gift was a T-shirt (Fig. 9.1b) and a ticket for an annual draw of fishing gears valued at 300 Euros. Three extra tickets were gift to the fishermen which give the meagre for biological analysis.

Furthermore, an automatic notification system was implemented and information of any meagre sale in the fish auction of the central fish market was obtained almost on line. Thereby, we could obtain information of some of the recaptured meagres that otherwise would remain non-reported or which have lost the external tag because had spent long time at liberty. These long-time recaptures were very important for improving precision and accuracy of mortality. Even, in some cases, these fish has been located before they were sold at the final customer. When possible, in these cases fish have been bought by us.

9.2.2 Estimating mortality

The survival probability of released hatchery-reared fish over time is assumed to be distributed in time following an exponential distribution (McGarvey, 2009).

For exponential models, Chapman (1961) proved that the reciprocal of mean recaptures at time (τ) by the finite sample size-correction factor (F) was the maximum likelihood estimate of total mortality rate (Z):

$$Z = \frac{1}{\bar{\tau}} F$$

$$\bar{\tau} = \frac{\sum_{i=1}^{n_T} \tau_i}{n_T}$$

$$F = \frac{n_T - 1}{n_T}$$

where n_T was the total number of recaptured fish.

Similarly, the standard error ($s.e.$) of the mortality was provided by:

$$s.e. = \frac{Z}{\sqrt{n_T - 2}}$$

Contrary to what may seem, this estimate is unbiased for non reporting tags. However, non reporting rate as well as mortality rate has to be assumed constant in time and in space (McGarvey *et al.*, 2009). Even, changing the relative proportions of natural and fishing mortality, given that the assumption of constant total mortality rate holds, had no effect on the mortality estimation. Furthermore, the model assumed that tag-recovery experiments have run sufficiently long that few or no further recaptures of tagged animals were expected, although good enough mortality estimates were obtained in truncated (ie., some fish are still alive) experiments when the monitored time was long enough (McGarvey *et al.*, 2009).

9.2.3 Simulation experiments

The accuracy and precision of the method for estimating the mortality rate (*Z*) in realistic scenarios for the meagre were evaluated by computer-simulation experiments.

9.2.3.1 Experiment 1

In the first experiment, we specifically focused in evaluating the effects of various known (simulation-assumed) constant rates of tag reporting (*R*) and various known (simulation-assumed) mortality rates (*Z*). Five tag reporting scenarios, with tag reporting rates of 0.1, 1, 5, 10 and 50%, and five mortality scenarios, with rates of 1, 3, 7, 10 and 50%, were tested. A total of 20000 released fish were simulated during 1000 days. Each pair of tested parameters was simulated 100 times and an average of the estimated mortality was calculated.

9.2.3.2 Experiment 2

A second experiment was carried out to test the accuracy and precision of the method in a right-side truncated experiment. For that, 20000 released fish were simulated during one year (365 days). A simulated tag reporting rate of 5% and a simulated mortality value of 1% were used. As in the first experiment, the estimated mortality was the mean of 100 simulations.

9.2.3.3 Experiment 3

In the third experiment, a left-side truncated experiment was conducted consisting in removing the captured fish from the first 90 days. The objective was to check the method against removing recaptures that may be inflated due to abnormal mortality that seems to occur during some time after releasing (see below). The mortality estimate of 20000 released fish from 90 to 1000 days, with tag reporting rate of 5% and a simulated mortality of 1%, was calculated and averaged from 100 simulations.

9.2.4 Recapture meagre data

All the collected data of meagre recaptures during the restocking program (see Appendix Table I.3) with known release and recapture date were included in the mortality estimation. The release date was known though the external tag, by the age when the otolith was available or by the length/weight just for a few very clear cases.

The recapture date was known though the interview of fishermen. Recaptured meagres with imprecise information were excluded of this analysis.

9.3 Results

9.3.1 Simulations

In the first experiment, under all assumed scenarios of tag reporting rate and mortality rate, estimates of total mortality showed no evidence of bias in almost all the cases (Table 9.1).

		Z				
		0.01	0.03	0.07	0.1	0.5
	0.001	0.0078	0.0327	0.0643	0.0872	0.4716
	0.01	0.0090	0.0310	0.0705	0.1022	0.4939
R	0.05	0.0099	0.0301	0.0683	0.0991	0.4985
	0.1	0.0094	0.0301	0.0697	0.0998	0.5011
	0.5	0.0099	0.0299	0.0697	0.0997	0.4990

Table 9.1.- Mortality estimates for different simulated tag reporting rate (R) and simulated mortality rate (Z).

Mortality estimates only seems to biased when tag reporting was very low, thus involving a few recaptured fish. In all the other scenarios, Z were properly estimated.

For a possible tag reporting rate of 5% and a mortality rate of 1%, the number of surviving fish was exponentially decreasing over time (Fig. 9.2a). Similarly, the number of recaptured fish over time (τ) decreased exponentially (Fig. 9.2b).

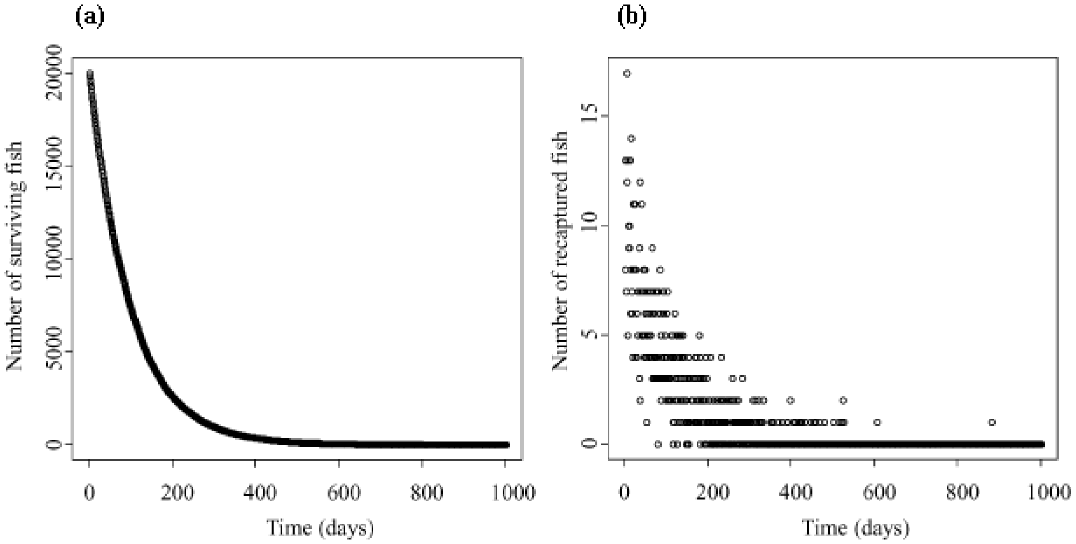


Fig. 9.2.- Simulation experiment 1 for a simulated mortality rate (Z) of 0.01 and tag reporting rate (R) of 0.05. (a) Number of surviving fish over time. (b) Number of reported recaptured fish over time.

In the second experiment, the simulation was conducted during one year only (365 days) to test if the method was able to estimate the mortality rate unbiased when the monitored period is shorter. The estimate mortality agreed closely with the true simulated mortality rate, *Z* = 0.0155 and *s.e.* = 0.0005, because after a year the number of surviving fish were very low (Fig. 9.3a) and the number of recaptured fish decreased considerably (Fig. 9.3b).

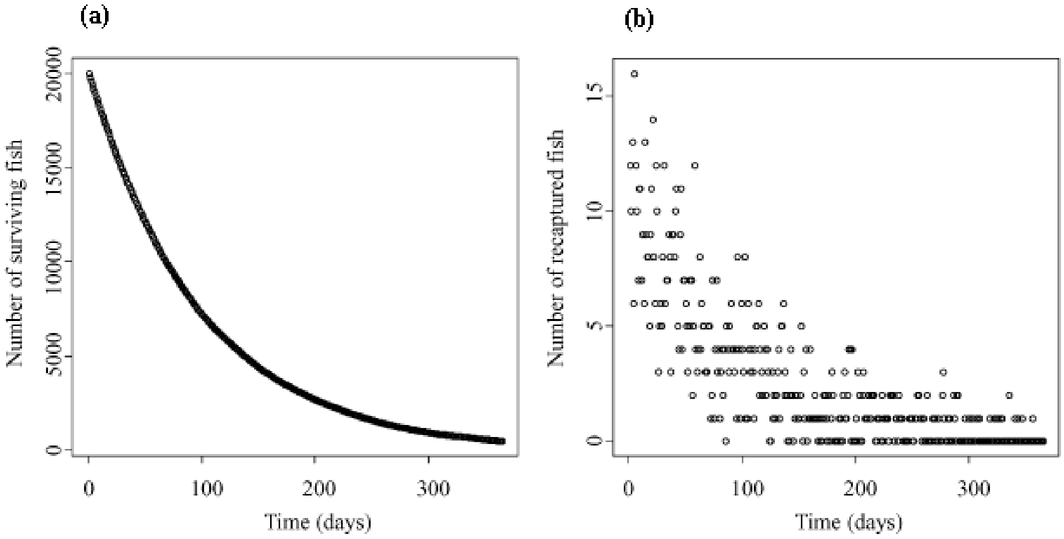


Fig. 9.3.- Simulation experiment 2 truncated after one year for a simulated mortality rate (*Z*) of 0.01 and tag reporting rate (*R*) of 0.05. (a) Number of surviving fish over time. (b) Number of reported recaptured fish over time.

The third simulation experiment showed that, despite deleting recaptures of the first 90 days (that is, the data was left-side truncated), the method continued estimating correctly the mortality rate. The mean of 100 simulations agreed closely with the true simulated mortality rate ($Z_{\text{true}} = 0.01$), showing an estimated mortality rate of 0.0098 and a *s.e.* of 0.0004.

9.3.2 Meagre recaptures

During the restocking program, a total of 13134 meagres (Appendix Table I.1) were released and 429 recaptured meagres were recorded, although just only 408 of these recaptured meagres had accurate information about the release and recapture date (see Appendix Table I.3). Therefore, the recapture rate of this study was 3.26%, although this rate showed a great variability between release events from 0-0.1% for release of smaller fish (Release #1, #3 or #4, see Appendix) to 23% or 14.1% for release of larger meagres (Release #16 and #13, respectively).

These 408 recaptures were compiled and used for the mortality estimation of released hatchery-reared meagre juveniles. The distribution of recaptured meagres over time presented an exponential distribution (Fig. 9.4).

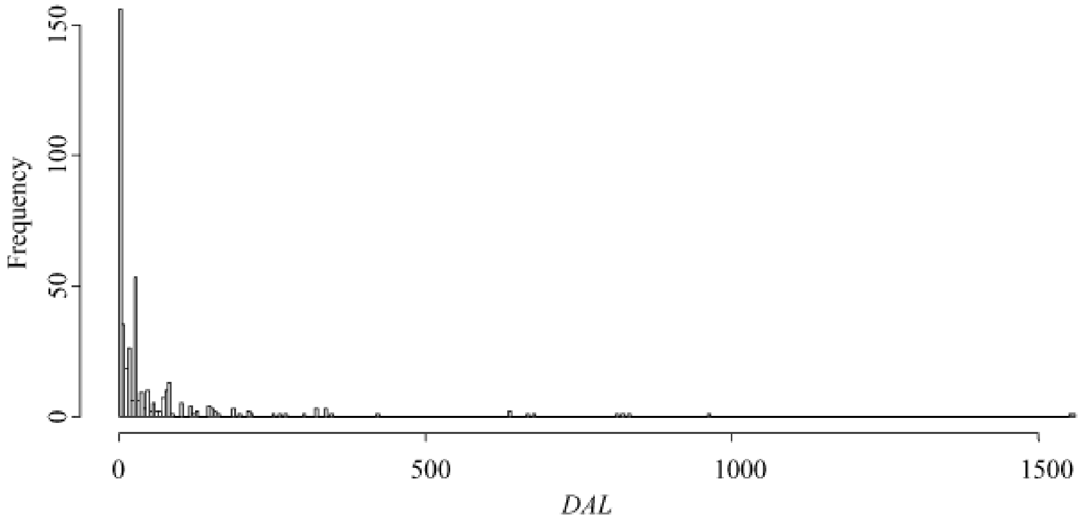


Fig. 9.4.- Frequency histogram of meagres recaptured over days at liberty (DAL). The width of the bar corresponded to 5 days at liberty.

The estimated mortality for the recaptured meagre distribution was 0.0169, i.e. 1.69% of the surviving meagres died every day, and the standard error of the estimation (*s.e.*) was 0.0008.

When a simulation (Fig. 9.5) were performed with the total released meagres (n = 13134) using the mortality rate estimated for the recaptured meagres (1.69%), a total of 26 surviving meagres remained after a year of release and there was none after two years. However, some meagres have actually survived more than two years at liberty. This fact suggests that this mortality estimate may be biased due to an increased and abnormal mortality during some time just after release.

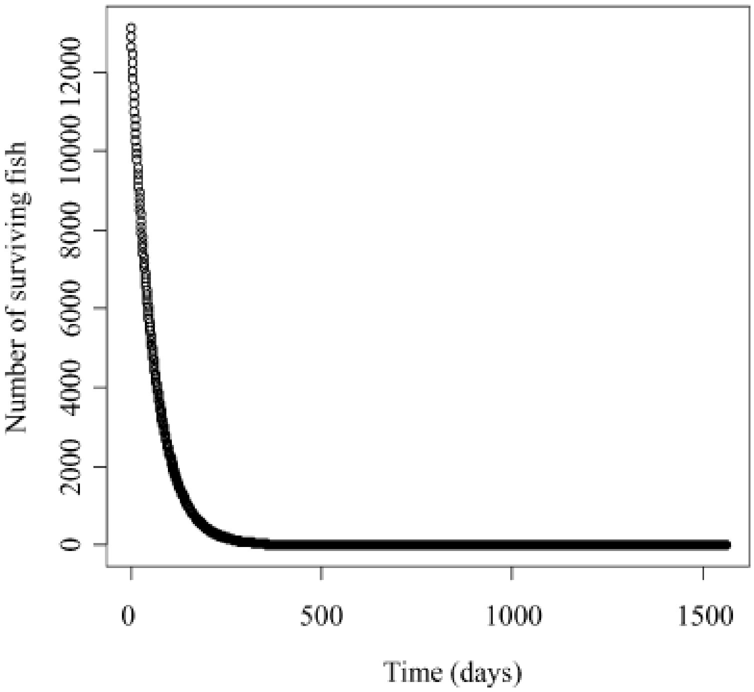


Fig. 9.5.- Estimation of the number of surviving meagres for the estimated mortality rate (Z) of 0.0169.

9.3.3 Mortality estimate after deleting the first 90 days

When the meagres recaptured during the first 90 days after release were deleted, the estimated mortality rate was 0.0029 and *s.e.* was 0.0004. Therefore, 0.29% of surviving meagres would die every day. Therefore, the mortality rate was lower than the observed for all the data set, indicating a shift in mortality a time after release. In that case, even the estimate of *Z* must be considered robust and unbiased (simulation experiment 3), it is more problematic to estimate the expected number of fish after two years at liberty. Also, it is problematic to estimate the date from which it is justified to assume that this shift in the mortality rate has been occurred. Only with illustrative purposes, the expected remaining fish after two years (i.e., when meagre reach sexual maturity) has been calculated for an increasing number of days near of the proposed 90 days (from 50 to 150). The results (Fig. 9.6) suggest that this number should be between 5 and 25. Nevertheless, it should be emphasized that this is an approximation. Contrasting, the expected number of surviving fish that experienced the same mortality estimated for fish returned from 90 days onwards (i.e., 0.0029) was as large as 2326.

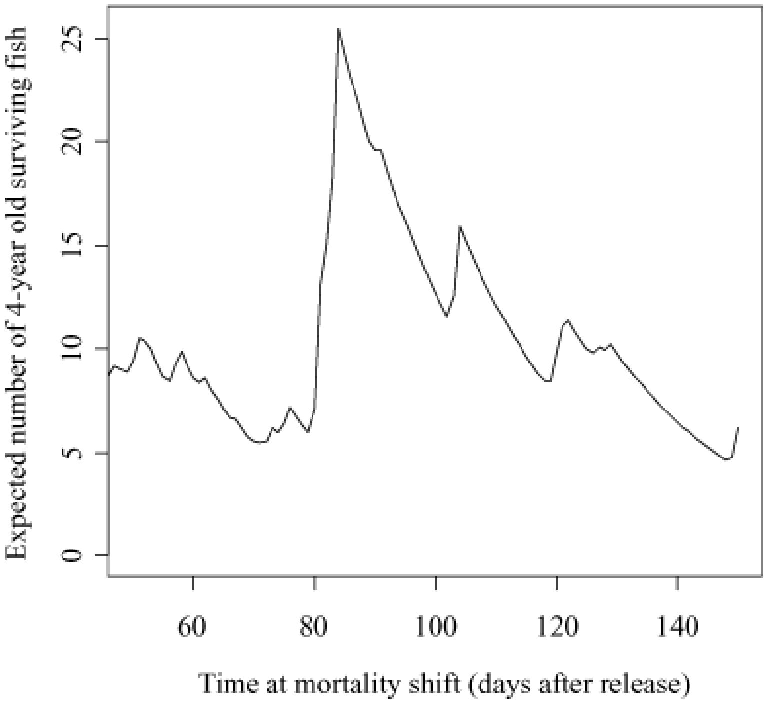


Fig. 9.6.- Expected number of 4-year old surviving fish for different moments of shift from short-term mortality to long-term mortality.

It is very interesting to remark that 10 returned fish was found to be sexually mature, thus empirically confirming that some fish may survive until sexual maturity, and thus that may reproduce at the wild.

9.4 Discussion

The restocking program for meagre in the Balearic Islands have demonstrated considerable success in developing spawning broodstock (Chapter 2), rearing juveniles (Chapter 3), tagging them (Chapter 4) and releasing them. However, the success of the program will depend at the end on how well the hatchery-reared meagres can adapt to natural habitat conditions and survive.

Over 13134 tagged hatchery-reared meagres were released in Mallorca island during the course of this study. The total recaptured rate was 3.26%, however a wide variability range were observed for the different release events. The largest meagres release (Release #13) presented a high recapture rate (14.1%) but a maximum recapture rate of 23% was presented in other large fish release (Release #16). Instead, recapture rate of 0% or close to 0% were observed when small fish were released (Release #1, #3 or #4, see Appendix). Clear evidences about the impact of the size-at-release on recapture rate have been proved, as was already reported elsewhere (Leber, 1995). A great variability in the recapture rate has been also reported in other studies, for example; from 0 to 31.3% for Atlantic cod (Svåsand *et al.*, 2000) or from 2.6 to 23.3% for white sturgeon *Acipenser transmontanus* (Ireland *et al.*, 2002), and the relevance of size-at-release were also well-established. This fact, as it has been detailed in Chapter 3, may be due to the greater impact of predation on smaller organisms (Tsukamoto *et al.*, 1989; Ray *et al.*, 1994) but also to the lower resistance to starvation in smaller individuals or to their poorer tolerance to environmental extremes (Sogard, 1997; Hurst & Conover, 1998; Lorenzen, 2000). However, as it has been commented in Chapter 3, higher size-at-release implies higher production and release costs and a reduction of the economic efficiency (cost per fish released). Therefore, the balance between cost-effectiveness and survival (i.e., success of the restocking program) needs a special consideration. Nevertheless, the relationship between recapture rate and size-at-release seems not to be linear, probably because predation-related mortality would tend to stabilize after overcoming a critical size (Leber, 1995; Jørgensen & Holt, 2013). After accounting for these factors, we propose that the optimum size-at-release for meagre could be around 35 cm, which corresponds to somewhat less than two years old, and a production cost of around 5€ fish^{-1} (Chapter 3).

In this study, post-release survival processes of hatchery-reared meagres were estimated through fishery tag-recapture data set using a maximum likelihood estimator. The precision and accuracy of the method were tested with the simulation experiments; however estimations could be biased by temporal and/or spatial changes in natural mortality or by gear selectivity for larger fish (Ireland *et al.*, 2002; McGarvey *et al.*, 2009). Both professional and recreational fishermen were not homogeneously distributed in the Balearic Islands because spatial-temporal patterns of fishing effort depend on weather, closures and restrictions, number of boats per port, port distance, accessibility or catch abundance (Morales-Nin *et al.*, 2005; Morales-Nin *et al.*, 2010b), although were necessary to assume constant for applying McGarvey's approach. Moreover, the selectivity of the fishing gear, mainly the trammel nets (responsible for much of the recaptures), could favor the capture of larger fish because the mesh does not allow small fish gill. The low recapture rate (almost zero) observed in the smaller meagres released could be due to having a too small size to be captured by the trammel nets. However, almost a year later, they would attain the size to be vulnerable to the trammel nets. The long-term non-appearance of fish released at small size strongly suggests that natural mortality suffered by these fish should be high.

The estimated mortality for the released hatchery-reared meagres was 0.0169, which means that 1.69% of the surviving meagres die every day. So, according with this estimate, after two years all the released meagres would be died. However, a few meagres that spent more than two years in the wild were recaptured, indicating that mortality may be overestimated. We propose that this may be related with an abnormally high mortality during some time just after releasing. In the same way, Hervas *et al.* (2010) proposed that released fish has both short- and long-term survival in the wild. This short-term mortality is conditioned by the adaptation to natural habitats and natural food sources and it has shown to be very high (Furuta *et al.*, 1997; Iglesias *et al.*, 2003; Watanabe *et al.*, 2006), reaching values of 66% per day in released turbot (Sparrevohn & Støttrup, 2007). Therefore, this short-term mortality can be decisive in the success of the restocking program. Optimizing the release strategies such as size-at-release, release habitat and microhabitat, release season, and density dependence, though pilot release experiments, can improve the released fish survival (Tsukamoto *et al.*, 1989; Willis *et al.*, 1995; Leber & Blankenship, 2011). Recent studies have demonstrated that conditioning or learning of reared fish before release could also improve behavioral skills such as feeding, burying and predator avoidance (Hossain *et al.*, 2002; Fairchild & Howell, 2004; Arai *et al.*, 2007), resulting in higher survival of juveniles just after release (Sparrevohn & Støttrup, 2007). However, only a few trials of pre-release training have been reported (Kanayama, 1968; Jarvi & Uglem, 1993; Berejikian *et al.*, 1999), because the widespread application of social learning protocols to train large numbers of fish in hatcheries is difficult (Brown & Laland, 2001). The results obtained for the adaptation capability of released meagres (Chapter 6) showed that they need a relatively log time (around 3 months) to recover their body condition index and shift to a more piscivorous diet. Therefore, the starvation period experienced by meagre just after release could cause a high mortality rate, thus threatening the success of the restocking program.

The meagres recaptured after two years of release were more than 3-4 year old and showed evidences of maturation in their gonads (Chapter 8), therefore, they were adults potentially able to reproduce at the wild. The success of a restocking program is determinate by the survival of hatchery-reared fish to sexual maturity in sufficient number to rebuild a sustainable age structure and provide the next generations of broodstock (Ireland *et al.*, 2002). However, precise estimation of this number is statistical more challenging than expected. Nevertheless, the approximate combination of the two (high and low) mortality rates, seems to suggest that this number should be rather small and much more effort should be done in reducing the high mortality experienced by meagre just after releasing.

SECTION VI – Chapter 10. Conclusions

Meagre released into the wild.

10.1 General conclusions

- Producing several thousands of meagres every year has been made possible by combining hormonal induction with GnRHa of wild broodstock and the development of an specific larval rearing protocol based on shifting from rotifers, *Artemia* sp. and, finally, to a commercial weaning diet.

- Size-at-release directly affects the survival of released fish but the release of larger fish entails higher production costs. The optimal size-at-release was estimated at 35 cm or nearly 2-years old fish. After comparing different diets, minimum cost for producing fish of such a size (around 5 € fish^{-1}) was obtained with commercial pellets, although maximum growth rate was obtained with a fresh fish-based diet.

- Developing an efficient tagging technique is an essential step in any restocking program because it facilitates the identification of released fish. All of the available methods have some pros and cons. We propose that double tagging with T-bar anchor tags and fluorescent ALR mark in the otolith allows detecting the recaptured meagres both on short and long term.

- Movement pattern of the released meagres shows that fish remains stationary at shallow sites during daytime, whilst at night fish seems to move following a random walk-like path, with relatively large and fast displacements, which allow them to reach any point around Mallorca in a short time. Conversely, dispersal off Mallorca is considered improbable.

- Concerning the adaptation capability of released fish, after around 3 months showing low stomach content and poor body condition, the surviving meagres seem to adapt to the new environmental conditions, as indicated by recovering a good body condition and shifting from an invertebrate-based to a more piscivorous diet.

- The growth curve of meagres that spent less than 3 months in the wild is similar or even lower than the expected growth during captivity. However, the growth of meagres that spent more than 3 months is higher than expected. We propose that this change is due to a greater energetic efficiency of piscivorous diet and to the differential survival of released meagres that have already a higher growth rate before releasing.

- In the Mediterranean Sea, meagre has a size at maturity (L_{50}) of 49.3 cm for males and 57.2 cm for females and an age at maturity (A_{50}) of 2.7 years for males and 3.5 years for females.

- An abnormally-high mortality during some time just after releasing can endanger the success of the meagre restocking program. However, adapted meagres have a higher chance of becoming adults, although few specimens seem to reach such stage.

10.2 Implications of meagre restocking program

Restocking is not simply releasing hatchery-reared fish into the wild. A responsible restocking program involves designing a fully-detailed restocking project, setting precise goals, completing a number of pilot experiments with tagged fish and, specially, evaluating the success of the release (Bartley & Bell, 2008).

Until recently, *Argyrosomus regius* has received little attention, with some core aspects of the species' biology remaining unknown (González-Quirós *et al.*, 2011). Through a number of pilot experiments developed within the meagre restocking program carried out in the Balearic Islands, most of these previously unknown aspects of the species' biology have been revealed. They include new knowledge on movement pattern, growth, diet and reproductive strategy. In addition, detailed data on the adaptation capability and survival of hatchery-reared released meagres have been produced. Therefore, assembling all this information together has allowed a proper evaluation of the success of this restocking program and has provided an scientific basis for designing a roadmap for future releases of, not only meagre at the Balearic Islands, but any other endangered marine species elsewhere.

The first bottleneck of many restocking programs is to produce a large number of juveniles for release into the wild. Fortunately, a well checked protocol is nowadays available for *A. regius* but it has been the outcome of several research projects. The development of this protocol dates back to 1999, when the IFAPA (Andalusia) and LIMIA (Balearic Islands) started the grow-out project of meagre "*Proyecto Coordinado de Plan Nacional sobre el Cultivo de la Corvina (Argyrosomus regius)*" which was funded by JACUMAR. The promising results obtained encouraged to submit a more ambitious proposal that covered the full cycle of the species. This second project entitled "*Plan Nacional de Cría de Corvina* (PLANACOR 2005-2007)" was funded by JACUMAR-SEGEPESCA. As a result of this project, the first meagre spawning in Spain was obtained in 2006 at LIMIA (Chapter 2), and a breeding protocol allowing the produce of large numbers of juveniles every year has been getting improved from such date onwards.

Restocking has been criticized because it may be neither effective nor economically viable (Bell *et al.*, 2006). Production costs are the most important component of the costs of a restocking project (Ungson *et al.*, 1993). Therefore, a third project "*Engorde experimental de corvina (Argyrosomus regius). Estudio de las patologías de engorde"* was completed, aimed to compare different diets in terms of both growth and cost (Chapter 3). The production of juvenile meagres until they reach the optimal size-at-release implies high costs, higher than those needed for growing other species currently used for stock enhancement (Svåsand *et al.*, 2000; Leber *et al.*, 2005; Patrick *et al.*, 2006). Nevertheless, in our case the objective went beyond stock enhancement because meagre disappeared from official landings statistics in the 60s in the Balearic Islands (Mayol *et al.*, 2000), where it can be considered extinct. Furthermore, meagre is a coastal marine top predator and, both terrestrial and marine, top predators have been extinguished from much of the globe (Myers & Worm, 2003). However, top predators play an important role in the ecosystems because they improve ecosystem resilience against globally-threatening processes such as biological invasions, disease transmission and climate change (Seddon *et al.*, 2007; Ritchie *et al.*, 2012). Therefore, meagre could have a great ecological value and a large investment in money and time to recover wild meagre populations in the Balearic Islands is justified.

Developing an efficient marking technique is an essential step in responsible restocking (Chapter 4) because it allowed the identification of released fish. Released fish that is captured and returned offers multiple information pieces, which have been used to analyze movement (Chapter 5), diet composition (Chapter 6) and growth (Chapter 7) of meagre. Furthermore, returned fish provide an objective way to evaluate how well the hatchery-reared meagre is able to adapt to natural habitat conditions and survive (Chapter 9) until they reach sexual maturity (Chapter 8).

In the case of meagre, success of the restocking program could be checked in the same way it has been defined for the white sturgeon (Duke *et al.*, 1999; Ireland *et al.*, 2002): (i) when hatchery production has restored a length and age frequency distribution in which most size and age classes are represented, (ii) numbers of adult spawners are sufficient to produce recruitment that maintains the population size and age distribution at a stable level, (iii) habitat improvements are sufficient to allow natural spawning to maintain the population in the absence of hatchery supplementation, and (iv) population size is sufficient to maintain genetic and life history diversity.

Until now, a wide distribution of length and age released meagres have been returned and some of them had reached sexual maturity. However, there is not direct evidence that meagres are spawning into the wild. Meagre forms large reproductive spawning aggregations (Quéro, 1985; Quéro & Vayne, 1987; Griffiths & Heemstra, 1995) but the minimum size of these aggregations needed for ensuring successful spawning to occur remains unknown. Given the noticeable short-term mortality (Chapter 9), it is possible the number of surviving adult meagres into the wild was insufficient to form viable spawning aggregations. Nevertheless, assuming that released meagres were able to follow innate cues, the high dispersion capability of meagre (Chapter 5) ensures that they could reach any proper spawning ground in Mallorca. Therefore, in order to increase the chance of spawning success, not only more hatchery-reared meagre juveniles should be released, but also post-release mortality should be improved. On the other hand, meagres present reproductive dysfunctions in captivity, i.e. fish undergo regular vitellogenesis, but with the onset of the spawning season the developing oocytes fail to initiate ovulation and instead they undergo atresic (Zohar & Mylonas, 2001). Therefore, the capability of meagre for aborting oocyte development in adverse environmental conditions may suggest that released fish could be also able to abort spawning when the environmental quality of the putative spawning grounds was poor. The availability of environmentally-adequate spawning grounds should be considered also as a key limiting factor for the success of a restocking program. This problem should be specially considered in Mallorca because the adequate habitats are especially prone to be affected by the tourism industry.

Nevertheless, hatchery releases should be dovetailed with other management measures to ensure resilient stocks with an appropriate spawning biomass, able to sustain optimum yields on a long term (Bell *et al.*, 2006). So, after obtaining spawning evidences of meagre into the wild, it would be desirable the establishment of complementary management regulations aimed to confer some additional protection to the released animals until such a resilient stock were successfully recovered.

Ultimately, this PhD has not only produced a broader knowledge regarding the biology of the meagre and best practices for restocking this species, but a generally applicable protocol for any responsible restocking program.

10.3 Future research

Research is a never-ending process. We identified some research lines stemming directly from our results that deserve further investigation. The large amount of information from acoustic tracking experiments, data storage tags and marking recapture data, allowed us to propose a conceptual model of the movement pattern of the released meagres (Chapter 5). However, recent advances in movement modeling (e.g., particle filtering; Royer *et al.*, 2005) suggest that these data can be combined in an explicit movement model. Complex models implying time-dependent shift in the movement style (for example, diel changes or spawning-related changes) are becoming plausible. Moreover, combination of spatial and/or temporal heterogeneity in mortality (resulting from heterogeneous fishing pattern) was unlikely just a few years ago. Nowadays, this objective is complex but may be a reliable challenge for a post-doc research. On the other hand, the survival estimations in Chapter 9 reflected that there may be a change in mortality rate when released meagres adapt to the new environment. Therefore, in-depth research on time-varying mortality models that were, at the same time, robust against non-reporting is another appealing research line for getting better estimations of the number of meagres that are currently able to reach sexual maturity.

Further, in so far as permitted by available funds, it should be recommendable to continue with the release of juvenile meagres in order to increase the number of adult specimens in the wild, thus increasing the chances of success. The difficulties presented by the hatchery-reared released meagres to adapt to new conditions (Chapter 6), and the consequent low survival (Chapter 9) suggest that some improvement should be made in the releasing strategy aimed to facilitate the adaptation of hatchery-reared fish. It is well known that training prior to the release and acclimation measures have caused positive results. Despite all this, logistic and economic difficulties would reduce the number of fish released; it may be more favorable to release few fish with high survival probability than to release many fish with low survival rates. Checking this hypothesis empirically will deserve more effort too.

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Appendix

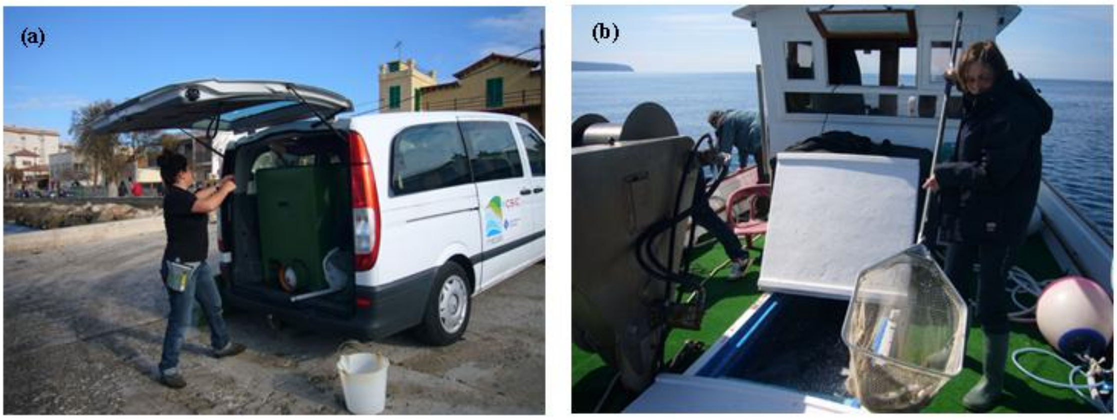

Fig. I.1.- Transports used for release meagre juveniles. (a) Overland with a van equipped with a transport tank 800 L and oxygen supplementation. (b) By sea in a boat equipped with a tank of 1000 L and oxygen supplementation

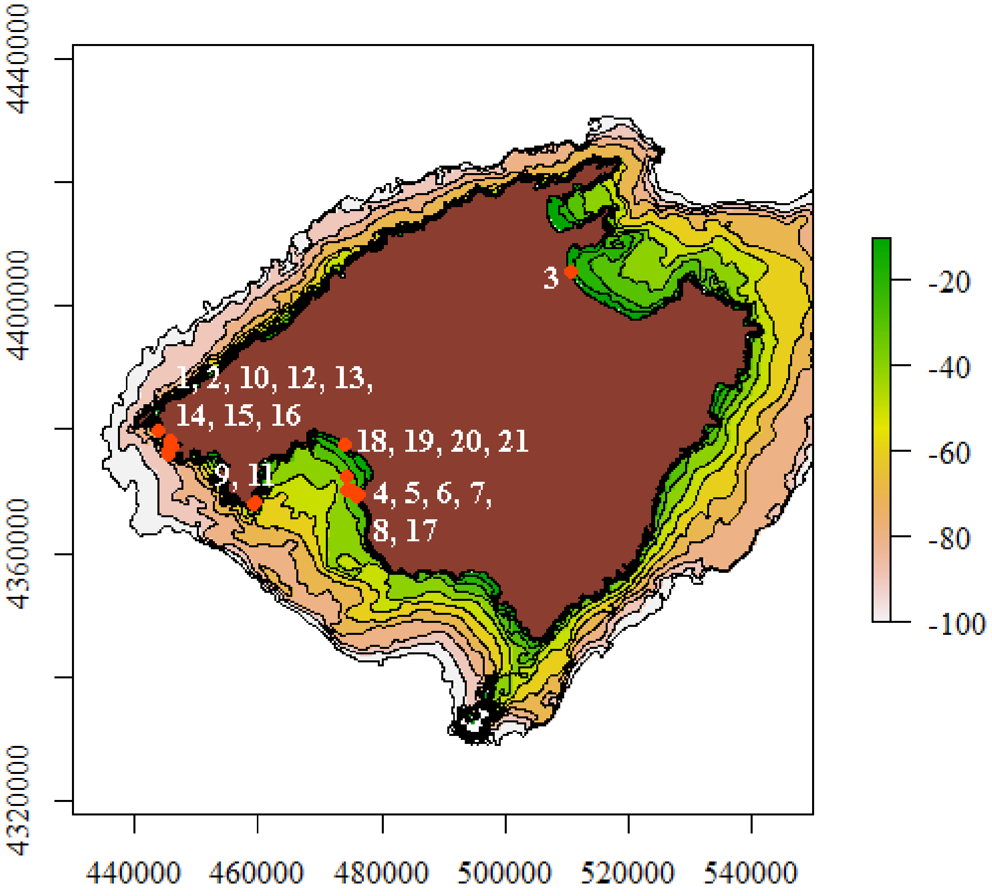

Fig. I.2.- Location of the 21 meagre release events in the Mallorca island.

Release	Relase Data	Birth	Age (months)	L_T (cm)	Num. specimens
1	05/11/2008	2008	6	15,3 ± 1,6	700
2	05/11/2008	2007	18	31,9 ± 2,0	208
3	07/11/2008	2008	6	15,6 ± 1,7	443
4	17/02/2009	2008	10	17,3 ± 2,17	2805
5	24/02/2009	2007	22	34,1 ± 2,48	1272
6	16/11/2009	2008	19	39,1 ± 2,1	170
7	18/03/2010	2009	11	23,6 ± 2,1	1009
8	16/04/2010	2009	12	25,5 ± 2,3	1018
9	21/09/2010	2009	17	32,9 ± 2,57	574
10	20/10/2010	2009	18	34,1 ± 2,6	224
11	14/12/2010	2009	20	35,7 ± 2,8	396
12	14/12/2010	2009	20	36,7 ± 2,5	397
13	16/12/2010	2008	32	51,5 ± 2,7	156
14	03/02/2011	2009	21	36,3 ± 2,67	397
15	18/05/2011	2009	25	45,6 ± 3,4	157
16	22/07/2011	2009	27	46,6 ± 4,0	126
17	20/09/2011	2010	17	32,3 ± 3,58	866
18	22/11/2012	2012	7	20,1 ± 1,97	1014
19	14/02/2013	2012	10	23,1 ± 2,3	510
20	14/05/2013	2012	13	25,2 ± 1,8	288
21	26/07/2013	2012	15	26,8 ± 2,7	404
				Total	**13134**

Table I.1.- Released meagres during the restocking program.

Total	Identified	Unidentified
429	408	21

Table I.2.- Number of recaptured meagres during the restocking program.

Release	DAL	Num. fish
2	15	4
2	16	4
4	9	1
4	18	1
4	271	1
4	336	1
5	1	10
5	2	31
5	3	23
5	4	3
5	6	3
5	7	4
5	8	1
5	12	3
5	23	1
5	25	1
5	27	1
5	28	2
5	29	3
5	32	2
5	35	2
5	43	1
5	47	1
5	50	1
5	56	2
5	61	1
5	80	1
5	81	3
5	114	4
5	150	1
5	156	2
5	161	1
5	212	1
5	264	1
5	322	1
5	340	1
5	350	1
5	422	1
5	667	1
5	812	1
5	822	1
5	833	1
5	961	1
5	1560	2
6	119	1
6	126	1
6	677	1
7	1	2
7	11	1
8	3	28
8	4	2
8	8	3
8	9	8
9	2	1
9	4	25
9	8	2
9	10	1
9	18	1
9	19	13
9	24	1
9	46	1
9	66	1
9	303	1
10	1	10
10	37	5
11	6	1
11	32	1
11	190	1
11	338	1
12	1	3
12	27	36
13	20	2
13	22	1
13	26	1
13	29	5
13	33	1
13	46	2
13	149	3
13	152	3
13	199	1
13	213	1
13	217	1
13	254	1
14	6	3
14	13	1
14	16	1
14	40	1
14	74	1
14	80	9
14	83	9
14	103	5
14	186	2
15	1	5
15	5	1
15	8	1
15	10	2
15	15	1
15	26	2
15	28	1
15	35	1
15	37	1
15	43	1
15	90	1
15	128	1
16	1	6
16	3	3
16	6	4
16	7	2
16	11	4
16	14	1
16	15	1
16	20	1
16	37	1
16	40	2
16	48	1
16	51	1
16	57	1
16	70	1
17	2	1
17	20	2
17	22	1
17	29	1
17	49	1
17	50	3
17	57	2
17	72	3
17	75	3
19	2	1
20	32	1
20	52	1
20	61	1
21	1	1

Table I.3.- Identified recaptured fish with their release number and corresponding days at liberty (DAL)

ID	Retention tag	Release	DAL	Recaptured L_T
R29	Untagged	4	271	44.1
R32	Untagged	4	336	46.3
R6	Tagged	5	1	35.4
R8	Tagged	5	1	37.0
R9	Tagged	5	1	34.8
R7	Tagged	5	2	32.5
R14	Tagged	5	2	35.2
R20	Tagged	5	2	30.0
R17	Tagged	5	4	35.8
R11	Tagged	5	6	35.4
R15	Tagged	5	6	34.3
R5	Tagged	5	7	34.3
R18	Tagged	5	7	36.7
R19	Tagged	5	11	33.2
R10	Tagged	5	12	37.1
R12	Tagged	5	12	34.6
R21	Tagged	5	29	31.2
R26	Tagged	5	29	38.0
R1	Tagged	5	30	34.0
R3	Tagged	5	32	30.5
R4	Tagged	5	35	34.8
R16	Tagged	5	50	32.2
R2	Tagged	5	56	34.2
R13	Tagged	5	56	35.6
R22	Untagged	5	81	31.1
R23	Untagged	5	81	34.6
R24	Untagged	5	81	35.2
R25	Untagged	5	81	38.6
R27	Untagged	5	156	39.6
R28	Tagged	5	156	40.1
R30	Untagged	5	264	51.6
R31	Untagged	5	322	53.6
R33	Untagged	5	340	65.6
R34	Untagged	5	350	52.7
R44	Untagged	5	422	67.9
R50	Untagged	5	822	79.1
R51	Untagged	5	833	66.9
R53	Untagged	5	961	71.3
R35	Tagged	6	119	42.5
R36	Tagged	6	126	40.1
R54	Tagged	6	677	77.0
R46	Tagged	8	7	31.2
R47	Tagged	9	18	35.2
R48	Tagged	13	21	55.6
R49	Tagged	13	22	52.7
R52	Tagged	15	43	49.0

Table I.4.- Recaptured meagres that were returned to the lab for biological analysis.

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