UNIVERSIDADE DO ALGARVE

Potential of fisheries restocking off the Algarve coast using aquaculture produced marine fish

Pedro G. Lino

Doutoramento em Ciências do Mar da Terra e do Ambiente
Ramo de Ciências do Mar
Especialidade de Ecologia Marinha

Tese orientada pelo Doutor Miguel Neves dos Santos (Investigador Auxiliar do INRB I.P./ IPIMAR) e pelo Professor Doutor Karim Erzini (Professor Associado da Universidade do Algarve)

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"Science never solves a problem without creating ten more."

George Bernard Shaw

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Resumo

A costa Sul do Algarve não é excepção à notória redução dos recursos pesqueiros que se vem verificando na costa continental Portuguesa. Cabe ao IPIMAR sugerir e testar novos instrumentos de gestão pesqueira que permitam melhorar o estado de conservação dos recursos pesqueiros, dado que as medidas tradicionais, como a limitação do tamanho das malhas das redes, do esforço de pesca ou a imposição de tamanhos mínimos legais de captura, se têm revelado insuficientes. Assim, o IPIMAR tem vindo a testar outras medidas complementares, tais como a criação de recifes artificiais ou o potencial do repovoamento, através da libertação de peixes produzidos em cativeiro. Tendo o conhecimento para produzir à escala experimental juvenis de várias espécies de Esparídeos, nomeadamente *Sparus aurata*, *Diplodus sargus*, *D. vulgaris* e *D. cervinus*, tornou-se possível testar essa medida de gestão na costa algarvia uma vez que a existência de estudos de repovoamento em outros países não invalida a necessidade de se realizarem experiências à escala local. Assim, este estudo teve como objectivo principal averiguar o potencial de repovoamento na costa do Algarve com peixes produzidos em cativeiro. Por outro lado havia a preocupação de perceber se a introdução de exemplares produzidos em cativeiro poderia ter um efeito genético negativo sobre as populações selvagens. Os resultados do estudo genético demonstraram que havendo uma boa gestão do conjunto dos reprodutores, não se verifica perda significativa de diversidade genética pelo que a libertação destes peixes não deverá afectar negativamente as populações selvagens. Os resultados obtidos através da marcação (convencional com marcas numeradas e telemetria acústica), indicam que a libertação de peixes nesta costa poderá ter efeitos positivos ao nível local, uma vez que as espécies testadas conseguem adaptar-se ao meio natural e que a sua dispersão se faz essencialmente ao longo da costa Sul do Algarve.

Palavras chave: Repovoamento, Esparídeos, marcação convencional, telemetria acústica, diversidade genética, peixe produzido e criado em cativeiro.

Abstract

The clear decrease in fisheries landings along the Portuguese coast and in the same scale off the south coast of the Algarve prompted IPIMAR, the Portuguese fisheries institute to test recovery measures for the stocks. In addition to restrictive measures such as mesh size, fishing effort or minimum legal size, it is possible to foster stock recovery with positive measures. Having created an artificial reef along the south cost of Algarve, IPIMAR proposed to investigate the possibility of stock enhancement by releasing hatchery produced and reared fish. Since IPIMAR already had the know-how to produce several Sparidae species, namely *Sparus aurata*, *Diplodus sargus*, *D. vulgaris* and *D. cervinus*, it was possible to conduct experimental tag and release trials with these species. Although similar studies have been carried out in other countries, it is a requirement that local species are tested at the local scale. Therefore the main objective of this study was to assess the potential of restocking the Algarve coast with hatchery produced fish. In addition, there was a concern that the release of hatchery produced fish could have a negative genetic impact on the wild populations. The results of the study show that if a good management of the brood stock is carried out, there is no significant loss of genetic diversity and therefore the release of this fish will not have a negative effect on the wild populations. The results obtained through several tagging methods from conventional numbered tags to acoustic telemetry, indicate that the release of fish off this coast could have a positive impact at the local level since the selected species are able to quickly adapt to the natural environment and the dispersion occurs mainly along the South coast of the Algarve.

Key words: Restocking, Sparidae, tagging, acoustic telemetry, genetic diversity, hatchery produced and reared fish

CHAPTER 1

Introduction and Objectives

Resource exploitation and fisheries management

Marine ecosystems cover the majority of the Earth's surface and are one of the most productive ecosystems in the world. These ecosystems provide essential goods and services for human wellbeing (Costanza et al., 1997; Wilson et al., 2005; Beaumont et al., 2007). Some of these goods and services are easily recognized as they are directly used by humans, such as food, medicines, fuel and energy, but also education, recreation and leisure (MA, 2003; Beaumont et al., 2007). Although equally vital for humans, others are less apparent, like gas and climate regulation, bioremediation of wastes, flood and storm protection, and nutrient cycling (Hiscock et al., 2006; Beaumont et al., 2007).

Fishing is the most widespread human activity in the marine environment (Jennings and Kaiser, 1998). Fish consumption per capita has been increasing steadily in the past decades, from an average of 9.9Kg in the 1960's to an historical maximum of 17kg per capita (FAO, 2010). This can be explained by several factors, namely by an increased concern about healthy eating, triggered by various food crises (e.g. BSE, dioxin), by the increased availability at supermarkets of prepared seafood based meals and by the improved economic situation and standard of living in some countries (Failler, 2007). As the world population has doubled in the same period, this means that the amount of fish captured or produced by aquaculture has quadrupled (Swartz et al, 2010). Since marine capture fisheries have been declining since the late 80's (Watson and Pauly, 2001) and over 80% of world's fish stocks are now considered to be fully or over-exploited (FAO, 2010), any growth in production comes from aquaculture. In fact, the reduction of the fisheries resources originating from capture fisheries has been compensated by the development of aquaculture. The aquaculture industry is undergoing a rapid worldwide expansion to fulfill the shortfall between the ever-

increasing world demand for seafood and decreasing availability of wild stocks due to the overexploitation and collapse of several fisheries worldwide (Gang et al., 2005; FAO, 2006; Worm et al., 2006). Aquaculture products accounted for only 4% of the total food fish supply in the 1970s (FAO, 2004), but have increased to 46% in 2008 (FAO, 2010). With an average annual growth rate of 6.9%, aquaculture is, nowadays, the fastest growing animal food-producing sector in the world (FAO, 2009). However, like fishing, which is probably the main anthropogenic driver of ecosystem alterations (by inducing changes in fish populations and communities, changes in the pathways of energy transfer and by disturbing and destroying the sea-floor habitats [e.g. Jennings et al., 2001; Choi et al., 2004; Zhang et al., 2009]), aquaculture may also cause adverse effects on the ecosystems, such as habitat modification and loss, organic enrichment, changes in biodiversity, eutrophication, chemical contamination, spread of diseases and parasites and introduction of exotic species (e.g. Cabello, 2006; Mente et al., 2006; Cao et al., 2007; Johnson, 2007; Cook et al., 2008; Cross et al., 2008; Holmer et al., 2002, 2008; Tett, 2008; Diana, 2009; Johnston and Roberts, 2009; Subasinghe et al., 2009). As a result of fishing and/or aquaculture activities, a wide range of ecosystems such as mangroves, seagrass beds, kelp forests, and coral reefs have been severely affected, leading to ecosystem changes and consequently to alterations in the services they provide. Since the degradation of marine ecosystems is so pervasive (Botsford et al., 1997; Jackson et al., 2001) in recent years, efforts have been made towards both the mitigation of fishing and aquaculture impacts and the restoration of natural resources, habitats and services (Gaspar et al., 2011).

We are currently in a situation where the over-exploitation of marine living resources and deterioration of the marine environment has reached an alarming level (Worm et al, 2009). Inversely, the production of new species with high fishing potential is growing at

an unprecedented pace (Bartley and Bell, 2008). Therefore, mitigation measures and restoration initiatives are needed aiming for better management of the marine environment and its living resources.

As mentioned by Santos et al. (2011) it is important to realize that traditional fisheries management measures (e.g. minimum sizes, closed seasons, catches limits, closed areas, and effort or gears restrictions) are insufficient for guaranteeing fisheries sustainability. These conditions generated a need to promote alternative and/or complementary management options directed to facilitating the sustainability of local artisanal fisheries. Among the different alternative measures for fisheries management, one that has reached general acceptance from both the fishing sector and managers is restocking. The establishment of restocking programs with specimens of target species, produced deliberately for this purpose and released in optimal areas for their development and survival, can benefit the fishing sector by mitigating resource depletion and contributing to the recovery of coastal fisheries.

Restocking

Restocking can be defined as the “deliberate release of fish or shellfish (mollusks and crustaceans) cultivated or wild with the intention of using the natural productivity of the release habitat” (ICES, 1994). This initial definition has been updated to mention that the goal of restocking is “to restore severely depleted spawning biomass to a level where it can once again provide regular, substantial yields”. If the goal is to “to increase productivity of an operational fishery by augmenting the natural supply of juveniles and optimizing harvests by overcoming recruitment limitation” then it should be named stock enhancement.

Capturing wild specimens and re-introducing them in areas of reduced abundance is a well tested practice but it has been abandoned due to the reduced results at recruitment time (Hoffmann, 1991). Restocking with hatchery produced and reared fish has several advantages: a high number of fish can be obtained from a small batch of adult fish (brood stock); juvenile mortality of cultivated fish is much lower than in the wild; timing and size of release into the wild can be selected to better match season or mismatch predators; stock recruitment is strengthened (Brown and Day, 2002).

The deliberate release of hatchery produced fish with the objective of using the natural production is carried out in many countries as a method to enhance fishing resources (Bartley, 1995; Munro and Bell, 1997). Restocking has become increasingly relevant in the last 30 years, being considered in several forums as one of the strategies to promote the sustainability of the fishing sector (e.g. International Conference on the Sustainable Contribution of Fisheries to Food Security held in Kyoto (FAO, 1995); in the Bangkok Declaration at the FAO Conference for Aquaculture (NACA/FAO, 2000); at the International Council for the Exploration of the Seas, as well as in numerous International fisheries and aquaculture Symposiums). Many countries have already established marine fish restocking programs. The Norwegian Sea Ranching Program has been responsible for the restocking of cod, salmon, alpine trout and lobsters since the early 80s (Svasand et al., 2000). The USA, Taiwan and China also have been carrying out restocking, in the case of Taiwan since 1978. But Japan is the country where restocking is more advanced. Since 1973 the Japan Marine Ranching Association (JASFA) has been the organization responsible for promoting restocking actions, with 75 centers, both public and private carrying out such activities (Katsuyama, 2000). When correctly applied restocking has the potential to increase stocks in the long term (Russell and Rimmer, 1997; Fushimi, 2001). In fact, it was demonstrated in the past that

stock enhancement effectively did lead to an increase in catches of several marine fish stocks such as *Mugil cephalus* in the USA (Leber and Arce, 1996), *Gadus morhua* in Norway (Svasand et al., 2000) and *Pagrus major* (Kitada, 1999) in Japan. According to Leber et al. (2004), in recent years 33 developing countries have reported the stocking of 59 marine or coastal species. Restocking is therefore an alternate and complementary tool for the regeneration of some fisheries resources with declining or depleted stocks. But this approach, in addition to the challenges inherent to the execution, is also extremely complex in terms of the analysis of the results obtained given the wide range of knowledge areas it involves. It is therefore necessary to have a multidisciplinary team since it aggregates knowledge from aquaculture production, genetics, biology and ecology, stock assessment and even socio-economics (Liao et al. 1999).

Like any other management action carried out by humans over a natural resource, restocking has several aspects that need to be considered:

1) Sanitary control: in order to prevent the transfer and establishment of diseases and parasites to the wild populations, a strict sanitary control is required which certifies the health of the organisms produced in aquaculture and later released into the natural environment. An accidental introduction of a pathogenic agent in the wild populations could compromise their viability, thus having the opposite effect of causing a reduction of catches due to the reduction of the natural population exploited (Caddy and Defeo, 2003).

2) Genetic control: the loss of genetic diversity has been observed both in wild populations of endangered species in risk of extinction and in organisms produced in captivity. Indeed, the nature and dimension of the habitat, and also fishing pressure (which reduces the number of specimens) may cause a modification in the genetic

structure of the populations of wild aquatic organisms (Taniguchi, 2003). In order to preserve the same level of genetic diversity present in the wild population, the selection and management of the broodstock are important aspects to take into account when doing a restocking action. Thus the introduction into the wild population of specimens produced in captivity from reproducers (generally in limited numbers thanks to the high fecundity of marine organisms) may lead to a reduction of the natural genetic variability due to the reproductive interaction between them (Smith and Francis, 1991). In addition organisms produced from genetically degraded progenitors have reduced capacity to respond to changes of the environmental conditions, which limits their capacity to survive in the natural environment. Although there is a need for rigid protocols to prevent undesirable genetic effects such as the translocation of exogenous genes e changes in allelic frequencies of the wild populations, such practices are not always respected (Ward, 2006).

3) Carrying capacity: another factor to take into consideration is the number of specimens to release into the environment. The amount to restock will depend on the target species' annual recruitment and on the habitat's carrying capacity, i.e. the number of specimens of a given species that the habitat is able to support at an optimal density which does not affect growth or survival (Bell et al, 2005). Although the carrying capacity is difficult to estimate, it is a critical factor for restocking programs and can vary according to the prevailing environmental conditions occurring in the season of the year the restocking is carried out: climatology, productivity of the ecosystem, currents, abundance of predators and competitors. The carrying capacity varies with location. Food availability is the main determining component and for species with highly selective diets it may be the limiting factor.

When the specimens released do not displace the wild population, it is a sign that the released number is within the carrying capacity of the habitat (Mustafa, 2003). Therefore it is important to have a preliminary study of the ecosystem, as exhaustive as possible, before carrying out any restocking actions (Bell et al, 2006).

4) Socio-economic: as mentioned previously restocking programs carried out around the world aim to mitigate the reduction in catches of a given resource as a response to overfishing, which makes a significant increase in landings the best indicator of success of a restocking action. However the simple comparison of catches (CPUE) before and after the restocking action might not be enough to evaluate the success since any positive effect might be masked by the natural inter-annual variability of the stock's abundance. Since the 50s researchers have been developing mathematical models to evaluate the results of restocking actions (e.g. the Jolly-Seber model), considering several parameters with mark and recapture being one of the key aspects. However there are not many studies that have proved the economic success of restocking actions and with a few exceptions (Fushimi, 2001) most did not return the expected results (D'Anna et al, 2004). On the other hand, for a restocking action to be considered economically attractive the cost of juvenile production must be as low as possible without compromising the quality criteria but the CPUE must also increase significantly (Lee, 1994; Moksness et al, 1998; Borthen et al, 1999). Considering these factors and optimizing the restocking management techniques regarding the biological knowledge of the species, behavior, carrying capacity of the habitat, age of the specimens, release season, and pre-adaptation to the habitat, several restocking programs carried out in Asia and Europe have produced positive results, reflected in increases in catches in posterior years (Rothlisberg et al. 1999; Su and Liao, 1999; Davenport et al. 1999; Jensen et al. 1994). In other cases the lack of

biological knowledge of the species or the absence of previous ecological studies in the restocking area caused the results to be less than expected (D'Anna et al, 2004). Most of the restocking programs in the past were carried out with public investment through government agencies (such as research institutes and universities) and non-profit organizations, without any justification to the general public. Additionally, although the profitability of a restocking action has a long-term return, the need to justify the investment of public funds with short term results led, on certain occasions to programs being abandoned precociously, thus precluding the opportunity to assess the benefits obtained (Travis et al, 1998).

In any case the fact that many restocking actions take place in areas where fishing activity is restricted makes professional fishermen consider these actions of little benefit and therefore they do not comply with the regulations necessary to make this tool successful. Therefore it is necessary to establish enforcement measures or better yet to involve the fishing community in order to make sure the rules are followed (Purcell, 2004). Nevertheless it is desirable that, in the particular case of over-exploited artisanal fisheries occurring in small littoral areas, the local fishermen associations (e.g. co-ops, producers guilds, and fishing clubs) in association with the technical assistance provided by the Administration and using a joint investment that includes aquaculture production companies, food processing companies, and traders, take the initiative to develop and enforce the restocking programs. This cooperation formula which ensures benefits for all participating parties is already being successfully used in Japan, Australia, New Zealand and USA (Masuda and Tsukamoto, 1998).

In the Iberian Peninsula a few experimental restocking actions have been carried out with several marine fish species. Since 1993 experiments were carried out using gilthead sea bream (*Sparus aurata*), senegalese sole (*Solea senegalensis*), white sea

bream (*Diplodus sargus*), among other species, in the Gulf of Cádiz (Southern Spain) with good recapture results (Sánchez-Lamadrid, 2002). Presently, the enhancement of local Portuguese fisheries has been based mostly on a program of artificial reef deployment, which began in 1990 in the southern (Algarve) coast (Santos and Monteiro, 1997, 1998). Currently, the Algarve artificial reef complex consists of seven large systems, which cover a total area of 43.5 km^2, and use more than 20,500 concrete blocks with a total volume of 100,000 m^3. Santos et al. (2011) summarized the most significant results of this program at the environmental and fisheries level. Thus, the National Fisheries and Marine Research Institute (IPIMAR), decided to conduct a series of experimental restocking studies, taking advantage of recent local developments towards the production of new and commercially important seabream species from the Sparidae family.

Aims and Objectives

The major aim of this study was to evaluate the potential of restocking in the Southern coast of Portugal, using three seabream species (*Sparus aurata*, *Diplodus sargus* and *D. cervinus*) produced and reared locally in aquaculture facilities. In order to achieve this, the following specific objectives were addressed:

1. Evaluation of tagging and release methods, assessment of retention, induced mortality and acclimation
2. Assessment of the genetic diversity of the fish produced
3. Evaluation of fish behavior and feeding after release in natural and artificial habitats;
4. Evaluation of residence time near release location and short and long term movements.

This thesis is organized in 9 chapters as follows: a general introduction and the objectives of the study are described in this first Chapter; Chapter 2 covers the technical aspects related with tagging, tag shedding and release methods; Chapters 3 to 7 correspond to 5 scientific papers covering the assessment of genetic diversity (Chapter 3) and evaluation of fish behavior (Chapters 4, 5, 6 and 7), feeding after release (Chapters 4 and 5), residence time near release location and short and long term movements (Chapters 4, 5, 6 and 7); Chapter 8 is the general Discussion and Suggestions for further studies; and Chapter 9 is a compilation of all the literature cited in all the previous chapters.

CHAPTER 2

Tags, tagging, release and monitoring techniques of hatchery produced and reared juvenile fish.

Introduction

The optimization of the tagging and releasing techniques is an essential task for the success of restocking trials since the appropriate methods should minimize tag loss (shedding) and animal injury.

In addition the size and the type of tags should be adequate to the size of fish released. Since it is one of the goals to optimize the size at release, it is essential to test the effectiveness of tagging (visibility, shedding) for different fish sizes and also to evaluate the effects of tagging on the fish (swimming performance, growth, rates, mortality).

Another important aspect of tagging that was assessed was the time period a tagged fish can hold the tag while held in tanks at high densities (during transportation to release site it may be necessary to concentrate fish up to 140 kg/m^3 for a period up to 2 hours). This is particularly important because after tagging fish need to be held in a limited space before they are released at sea. Since restocking trials were carried out off-shore, the weather conditions can prevent the release for days and sometimes weeks even during a gentle season. Therefore it is particularly important to know for how long fish can be held in such conditions and what is the amount of tag shedding.

The main objective of this part of the work was to get familiarized with the most appropriate tags and techniques of tagging, releasing and monitoring hatchery produced and reared juvenile fish. In order to achieve it, several pilot essays and one experiment were conducted. What follows is a brief description of these essays and study carried out and major findings achieved.

Conventional tagging

After a careful analysis of available bibliography and taking into account the species selected for tagging the T-bar anchor tags types FF94 and FD94 from FLOY TAG & Mfg, INC were chosen as being the most appropriate for the study's objectives. The characteristics of the two tag types are described in Table 1 and Figure 1.

Table 1 - Characteristics of the tags, tagging guns and needles used in the conventional tagging experiments.

Model (FLOY T-BAR ANCHOR TAG)	**FF 94**	**FD 94**
Filament material	P*olyolefin* monofilament	
Tubing material and protection	*Polyolefin*	
Filament length	10 mm	20 mm
Filament diameter	0.2 mm	0.5 mm
Tubing length	32 mm	37 mm
Tubing diameter	1.7 mm	2.0 mm
Tag weight	0.1 g	0.2 g
Tagging gun type	Pistol grip	
Tagging gun model	Mark II fine fabric	Mark III regular
Needle type	Fine fabric needle	Regular needle

Figure 1 - Tagging sea breams with a Fine Fabric pistol grip tagging gun (left) and a Regular tagging gun (right) (Image source: © IPIMAR)

Both tags have in common that they had printed the basic information (Figure 2) required to uniquely identify the fish (a unique number), as well as the name of the organization and country to be contacted and a phone number.

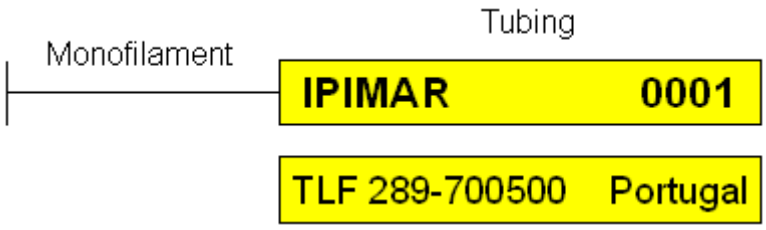

Figure 2 - Essential data printed on the tag consists of a unique number, organization name, country and phone number (Image source: © IPIMAR).

Breeders

The gilthead sea bream juveniles were obtained from a batch of breeders, captured from the wild from the Algarve's coast, with an individual weight between 0.5 and 4 Kg. The

white sea bream juveniles were also obtained from a batch of wild captured adult fish with weights between 0.4 and 0.8kg.

The feeding regime of the breeders of both species consisted mainly of squid (*Loligo* sp.) and sardines (*Sardina pilchardus*), fed ad *libitum* during the morning. This diet was complemented with feed optimized for gilthead sea bream breeders (PROAQUA) supplied in the afternoon.

Juveniles

The gilthead and white sea bream juveniles were produced at IPIMAR's Aquaculture Station in Olhão, and were reared in 3m3 fiber glass tanks, in open circuit regime with a daily water renewal frequency of around 10 times. The physical-chemical parameters were controlled daily, with temperature and salinity varying according to the natural environment of the Ria Formosa coastal lagoon and oxygen levels maintained at 5-8 mg/l using forced aeration. The photo-period was the natural and densities were variable (between 5 - 15 kg/m3) according to fish growth. The diet supplied to juveniles consisted exclusively of feed for gilthead “AQUASOJA” and “OPT-mini” continuously supplied by automatic feeders.

Tagging process

Before tagging fish were captured from the growth tanks and placed in small plastic tanks with forced aeration containing a solution of 0.2ml/l phenoxyethanol in sea water (Figure 3).

Figure 3 - Anesthetic bath containing phenoxyethanol. (Image source: © IPIMAR)

Anesthesia is essential in this process since it reduces stress, handling time and therefore risk of injuring the fish. When fish were lightly anesthetized (loss of equilibrium), they were weighed and measured (fork and total length) and then tagged.

The needle was inserted in an oblique axis to the length of the fish in order to reduce drag during swimming and therefore minimize the damages to the skin in the insertion area. According to the instructions from the manufacturer, the tags were inserted on the dorsal area specifically under the first rays of the dorsal fin, as shown in Figure 4. After tagging fish were placed in a recovery tank with clean highly aerated sea water in order to accelerate anesthetic dilution and recovery. Once the fish were fully recovered they were placed back in the $3m^3$ growth tanks for at least 3 days, and provided with prophylactic antibiotic treatment

Figure 4 - Tagged white sea bream (*Diplodus sargus*) recovering from anesthesia (Image source: © IPIMAR)

Assessment of tag retention

1) Comparison of two tag types

In this experiment 6 batches of 30 fish were tagged, three with T-bar anchor model FD94 and another three with T-bar anchor model FF94. One batch of each tag type was placed in three replicate 600 l fiber glass tanks. The number of tags lost as well as the incidence of injuries was registered for two months. The characteristics of the 6 batches are described in Table 2.

Table 2: Characteristics of the white sea bream batches used to assess comparative tag shedding using two Floy Tag models. A, B and C are the 3 tanks. Batches 1, 3 and 5 were tagged with the larger FD94 tag while Batches 2, 4 and 6 were tagged with the smaller FF94 tag.

Tank	**A**		**B**		**C**	
Characteristics / Batch	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Tag type	FD94	FF94	FD94	FF94	FD94	FF94
Number of fish	30	30	30	30	30	30
Total number of fish	60		60		60	
Total weight of fish (kg)	2.384	2.609	2.759	2.369	2.515	2.494
Density (kg/m3)	8.322		8.457		8.348	
Minimum fork length (cm)	12.6	12.7	12.3	12.0	11.9	12.9
Average fork length (cm)	15.0	14.5	14.8	14.1	14.5	14.4
Maximum fork length (cm)	18.7	15.9	19.1	15.6	17.7	16
Minimum weight (g)	50	68	49	59	55	58
Average weight (g)	91.2	87.0	92.0	89.0	86.7	83.1
Maximum weight (g)	160	110	173	100	153	118

The results obtained showed that there were no significant fish size differences between the 3 replicate tanks (One Way Repeated Measures ANOVA, Power=0.378, P=0.058). The fork length class distributions of the 3 tanks are displayed in Figure 5. There were also no significant differences between the size distribution of fish tagged with small

(FF94) tags and large (FD94) tags (Mann-Whitney Rank Sum Test, U= 3367.500, P= 0.066), so the only factor affecting tag loss is the type of tag.

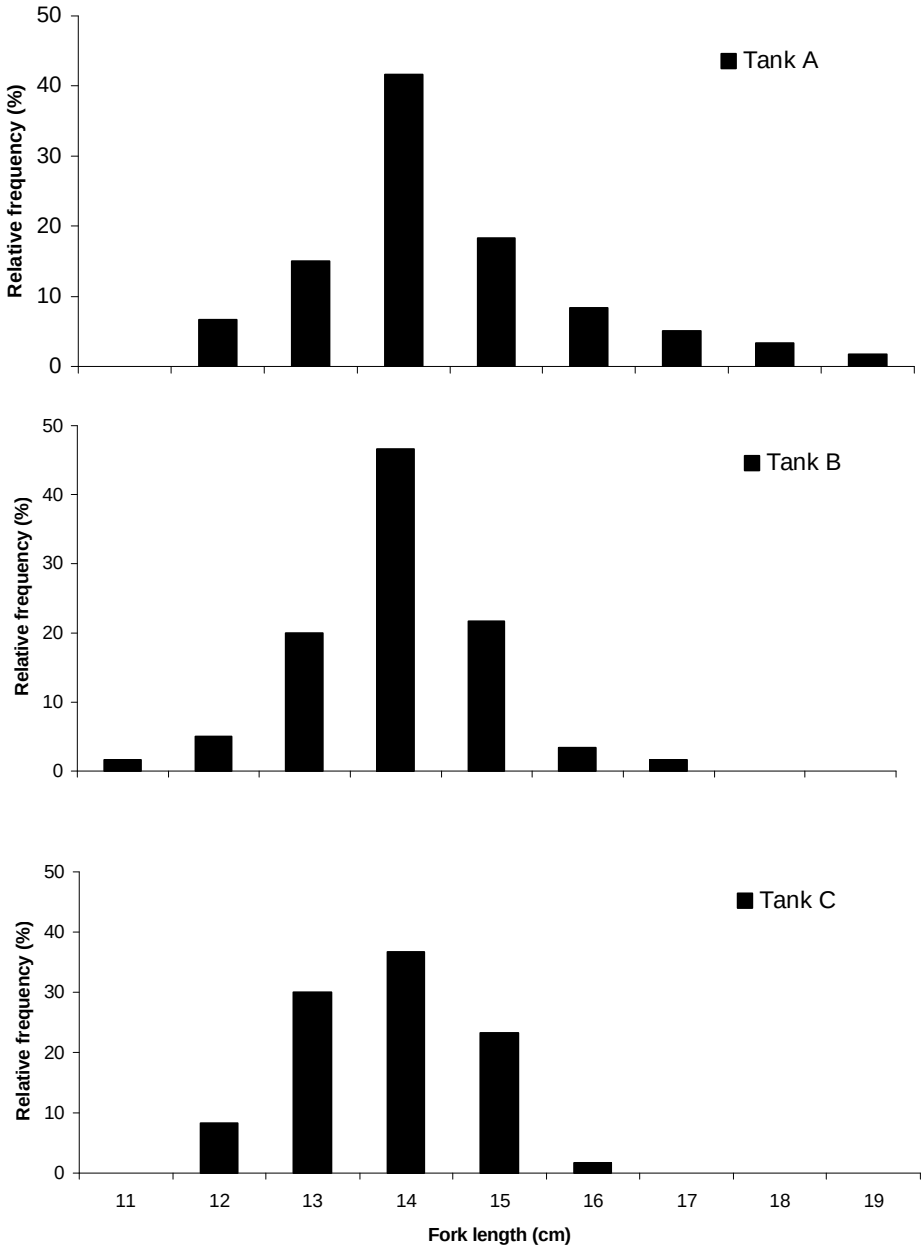

Figure 5 - Fork length frequency distributions of the three batches of white seabreams (*Diplodus sargus*) used to test tag retention.

As can be observed from Figure 6, tag loss was extremely limited (less than 10%). It is also worthy of notice that there was no tag loss before day 23 in any of the replicates. This means that tagged white sea bream can wait in a holding tank without any tag loss for over three weeks period. In addition, after 49 days, in total only 9 out of 90 (10%) FF94 tags and only 5 out of 89 (6%) FD94 tags were shed. This means that there was no statistically significant differences in tag loss (z-test, z=0.813, P=0.416). Since larger tags are easier to handle, this model was preferred. There was no mortality associated with the manipulation of fish or due to tagging. This was also an extremely important result.

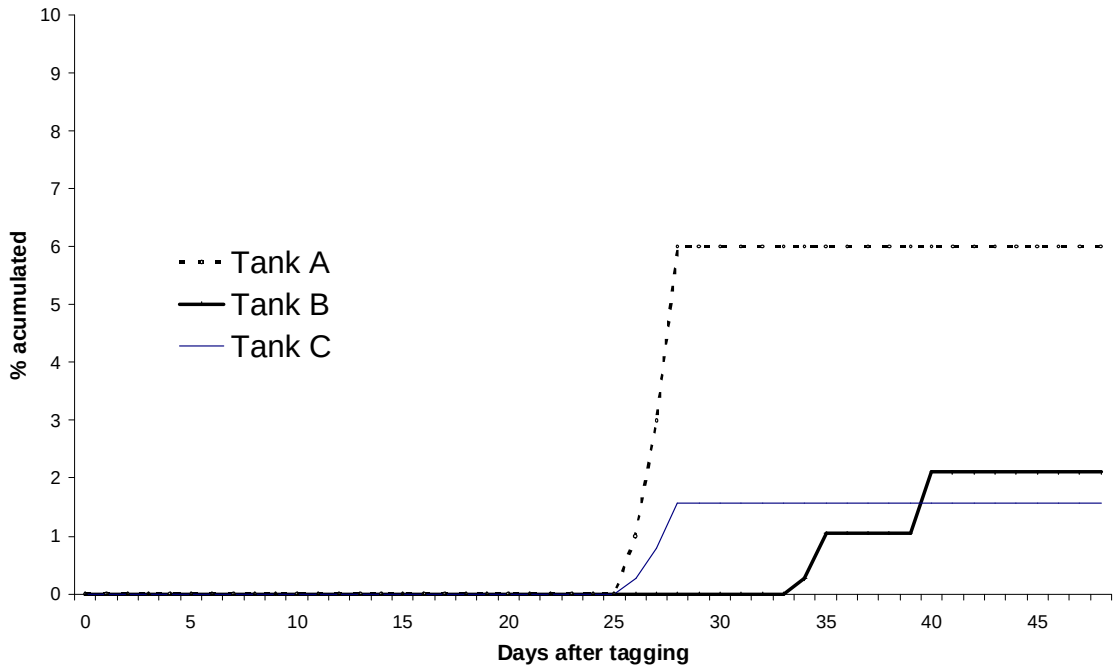

Figure 6 - Cumulative tag loss of white sea bream (*Diplodus sargus*) over time

2) Comparison of tag loss at different high densities

The main goal of this experiment was to assess how long fish could be stocked at high densities after tagging. To test this hypothesis gilthead seabreams (*Sparus aurata*) were tagged with T-bar anchor tags type FD-94 and kept in two tanks at very high density (more than 20Kg/m^3).

The characteristics of the fish in the two batches are described in Table 3. The experiment lasted for 48 days and the number of lost tags was registered on a daily basis.

Table 3 - Characteristics of the 2 batches of gilthead sea bream (*Sparus aurata*) used to test tag retention at high densities.

Characteristics	Batch 1	Batch 2
Tag type	FD94	
Number of fish	373	430
Total number of fish	803	
Total weight of fish (kg)	150.96	162.31
Density (kg/m3)	25.2	27.0
Minimum fork length (cm)	20.8	21.3
Average fork length (cm)	25.8	25.6
Maximum fork length (cm)	36.6	29.9
Minimum weight (g)	202	198
Average weight (g)	407.7	377.5
Maximum weight(g)	866	573
Age (days)	765	709

The length distributions of the two samples used are shown in Figure 7. There were no statistically significant differences in size distribution between the two tanks (Mann-Whitney Rank Sum Test, U= 58.000, P=0.895) so the only difference was the density. The difference in density could explain the faster rate of tag shedding (Figure 8) since at a higher density there are more interactions between fish and it can be easily established from the damaged state of the recovered tags that the cause for tag loss is reciprocal pulling off the tags (Figure 9).

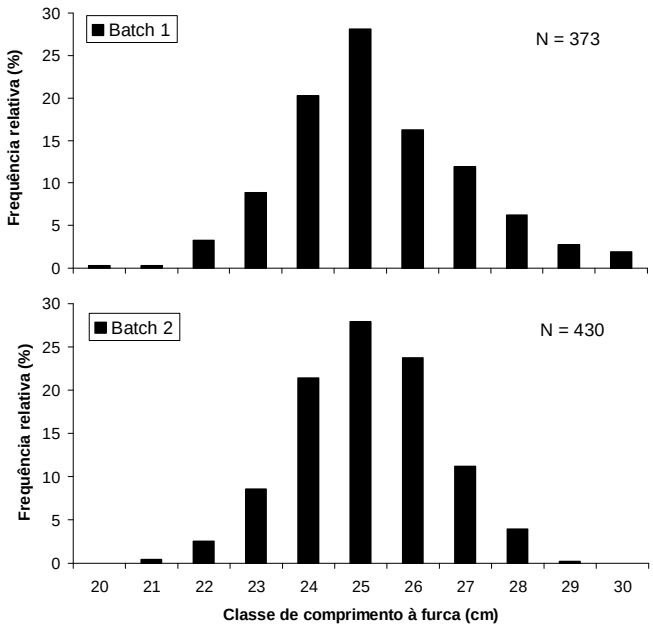

Figure 7 - Fork length frequency distributions of the two batches of gilthead sea bream (*Sparus aurata*).

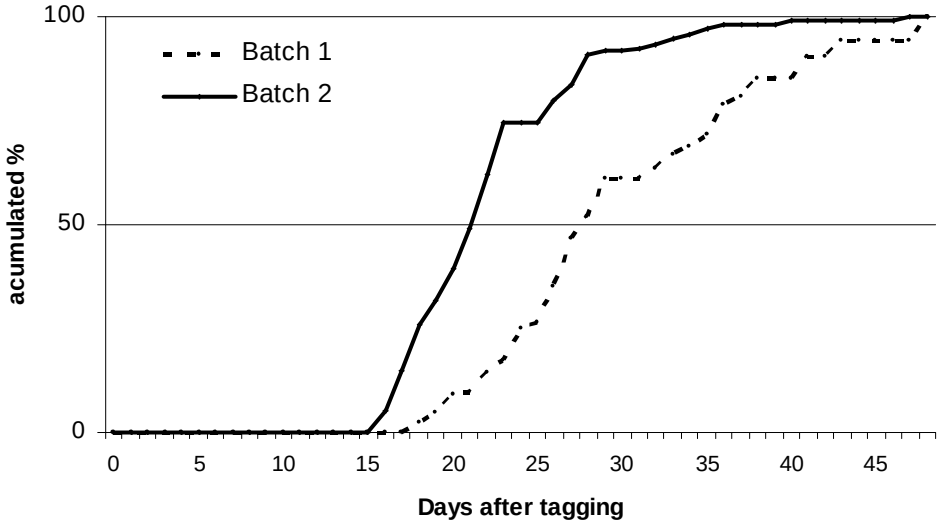

Figure 8 - Evolution of the percentage of tags lost (shedded) with time, in two gilthead tanks with densities of 25.2 and 27.0 kg/m^3.

It is also important to note that there was no tag loss before 15 days, so this seems to be the limit for holding gilthead seabreams at these high densities. Although the results were not directly comparable (since the *D. sargus* densities in the previous experiment were much lower), the observation of the tags lost in the *D. sargus* experiment never showed similar bite marks. This suggests there is a behavior that can be attributed to *S. aurata* and may also happen at lower densities if tagged fish are kept for long periods.

Figure 9 - Detail of the condition of the T-bar anchor tags after being mutually pulled off by gilthead seabreams. (Image source: © IPIMAR)

VIE (Visible Implant Elastomer)

This non-conventional method was used during this study for an experiment with tagging and releasing very small sea bream juveniles (smaller than 10g).

VIE is a silicone compound that is biocompatible medical grade material. This means that it is not toxic. It is provided as two separate liquids which are mixed and can be injected in visible parts of the fish. The mixed liquid will become a solid flexible silicone marking within an hour or less (depending on temperature).

In addition the VIE pigment can be fluorescent which has the advantage that even if the tagged location becomes less transparent as the fish grows, it can still be detected by eye, using the manufacturer's supplied VI light (a LED based "black light" - BL).

The major advantage of this method is that it allows tagging of very small fish, which in theory would be the ideal target for a massive restocking, since it would reduce production costs. In addition, due to the extremely small amount used in each fish (as little as 5 microliters) it is an extremely cost effective method. Obviously it has a great limitation compared to conventional tags: it is not clearly visible (and therefore fisherman will not return them since it is not noticeable). Furthermore, even if the fisherman notices it, he will not have any information on the tag to act upon.

VIE is usually injected into transparent adipose membranes like the ocular membrane of a trout. Sparidae do not have such membranes and therefore a suitable location had to be found. After several tests it was found that the best location to use this tag is in the caudal fin in the membrane that connects the fin rays (Figure 10).

Figure 10 - Fluorescent green VIE tag injected into the caudal fin membrane.

A short pilot survival experiment was carried out with 3 white seabreams (*Diplodus sargus*) and 3 common two-banded seabreams (*Diplodus vulgaris*). After 3 months in

an aquarium, no mortality or tag loss occurred and the tag was still clearly visible using the BL flashlight.

Release techniques

For batch release of conventional tagging two types of release methods were tested: release at depth (using a cage or a PVC tube) and release at surface (using a dip net, tipping the tank). The comparison of these methods allows the selection of the most efficient releasing method, to minimizing tag shedding and maximizing fish welfare and residence time in the release area.

Comparison of release methods

1) Releasing at depth

A batch composed of 1,000 gilthead sea bream (average 200g) was tagged using T-bar anchor tags model FD-94. Fish were transported in two fiber glass tanks of 1.5m^3 with constant water renewal (open system) and oxygen supplementation.

The cages used consisted of a metal frame covered with a plastic mesh (Figure 11), with a top opening door. The characteristics of the cage are described in Table 4.

Figure 11 - The transport cage in the water tied to the side of the boat. Fish are placed inside through the top opening. (Image source: © IPIMAR)

Table 4 - Characteristics of the cages used to release fish at depth in the stocking experiments.

Parameters	Characteristics
Frame	Iron
Height	80 cm
Width	120 cm
Length	120 cm
Volume	1.152 m3
Mesh cover	Plastic
Mesh size	30 mm

Before placing the fish in the cages, these were lowered to sea level and tied to the boat structure for the duration of the fish transfer. Initially the fish were collected with a dip

net from the transport tanks and placed in the cage. Posteriorly this method was optimized using the PVC tube with a funnel, with fish being collected from the transport tank with a dip net, placed into the funnel and allowed to gently slide down into the cage. Using the PVC tube further reduced handling time and damage to the fish.

In spite of all the optimization this is still a delicate and slow process since handling can cause damage to the fish and even tag loss (this was observed only for tags FF94).

When the cages were full, they were slowly lowered to the sea bottom by SCUBA divers, who check on the fish condition. This process was also slow to allow fish to accommodate to the increased water pressure. Since the fish used in this study were hatched and reared in captivity, they have never been as deep as the artificial reefs (located at 20m depth) and subject to a pressure of 3atm.

When the cage was positioned on the sea bottom near the artificial reefs, the door was opened by the diver and the fish swim freely out (Figure 12). All fish seemed to be in good condition and there was no mortality recorded.

Figure 12 - Tagged white seabreams (*Diplodus sargus*) swimming freely out of the transport cage at 20m depth.

PVC pipe

The PVC pipe used for this method is composed of several sections 6 meter long and 16cm in diameter. The sections can be fitted one into the next until the desired length is achieved. The free end was tied to the artificial reef structure by SCUBA divers to maintain position.

This system is quite easy to handle at low depth but extremely difficult at 20m (depth of the artificial reefs) due to the accumulated weight and the resistance to any current. In addition steering the boat to maintain an exact position can only be done under very calm sea

After the free end was tied to the artificial reef, fish were placed in the tube opening. By using a water pump a down current forced the water out at depth (Figure 13).

Figure 13 - Placing fish in the PVC tube for the release method trials.

This method would have the advantage of reducing the fish manipulation (compared to the cage method) and placing the fish in the tube was quite fast. Unfortunately it proved to be inefficient since the rate of exit at depth was extremely slow (Figure 14) and the fish started to clog the tube, so the tube had to be dismantled (the fish were released in mid water instead of near the artificial reefs). In addition releasing the fish with a time lapse might prevent the aggregation in a protective school. The system proved to be efficient only at shallow depth.

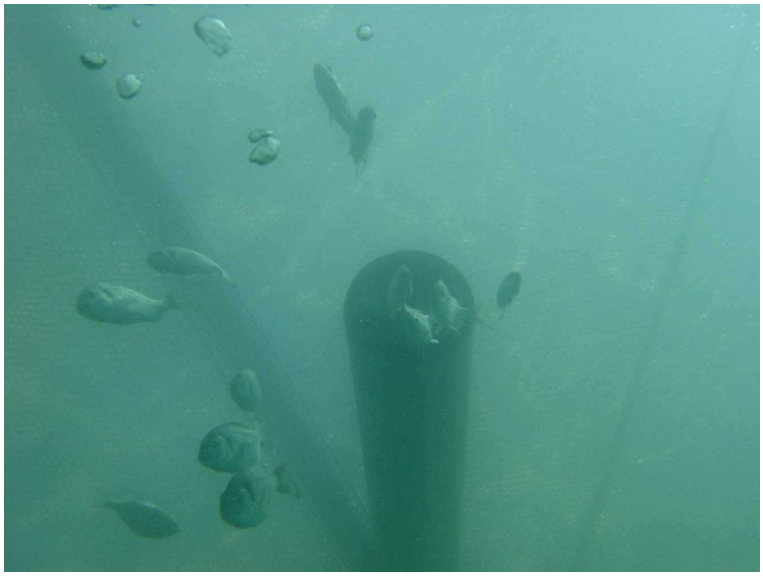

Figure 14 - Fish exiting at the free end of the PVC pipe.

2) Fish Release at the surface

Release at surface with a dip net

For releases of fish in shallow water a simpler method was used. Fish were placed directly in the water using a dip net with a long handle. This method worked extremely well, fish were in excellent conditions and no mortality neither shedding was noticed. A clear disadvantage of this method is that fish are released in small batches instead of a school.

Figure 15 - Releasing fish at the surface with a dip net wit a long handle.

Release at surface with holding tank

Another method of releasing fish at low depth tested was by simply dipping/tipping the holding tank. This method proved to be extremely efficient, with no fish manipulation at all and therefore no stress for the fish: Moreover, it was extremely fast to carry out. An additional advantage was that fish were released as a large school, which seemed to be a good way of promoting aggregation. This method has the limitation that it can only be carried out in shallow waters and with extremely calm sea state.

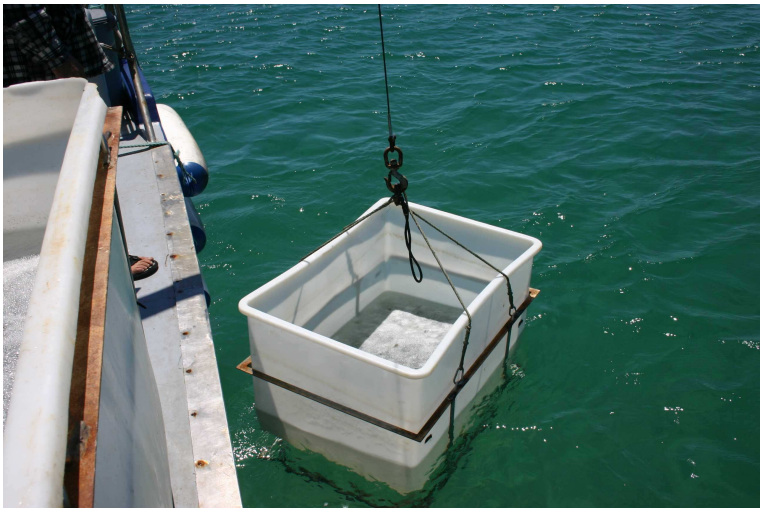

Figure 16 - Releasing tagged fish at the surface by submerging/tipping the holding tank.

In conclusion, the most appropriate methods for a restocking action at depth was the submerged cage, in spite of the increased operational costs and when releasing at the surface, tipping the transport cage was the most efficient.

Acoustic telemetry

Underwater acoustic telemetry in the sense of this study was used to monitor the presence and movements of fish remotely. The equipment used for this component of the study is the most widely adopted, produced by the Vemco company. It is relatively inexpensive but it is also limited. It transmits and receives in a single frequency (69 KHz) and the receiver only detects the presence of a tagged fish, recording the time and the time of detection. The sounds transmitted are coded so that each tag transmits a unique ID and in theory up to 256 fish can be correctly identified in the same area.

The transmitter is a small capsule that can be placed outside or inside the fish. It can transmit continually or at user select intervals. Each time the transmitter sends a sign (called a ping) it drains the battery. So for a given battery you can choose a frequent ping which will provide a lot of detections in a short period or a spaced ping which will allow a longer study period.

In order to avoid modifications in equilibrium, swimming performance and behavior of the fish, it is generally accepted a “rule of thumb” is that the weight of the tag should not exceed 2% of the fish weight.

This has two implications: in spite of the miniaturization of the components it is not possible to tag very small fish; and the duration of the study is conditioned by the size of the fish selected (and vice-versa).

Surgical implant of the tag

Based on the experience of previous studies with acoustic telemetry using white seabreams in Italy, in the current study the tags were also implanted internally. In order

to implant the tag in the fish organ cavity a small surgery was performed. The fish was anesthetized in a 60 l tank container with a solution of 0,4 ml l-1 2-Phenoxyethanol.

When the fish presented loss of equilibrium and a slower breathing rhythm (meaning it had reached stage 3 of anesthesia), it was placed upside down in a V-shaped berth (Figure 17).

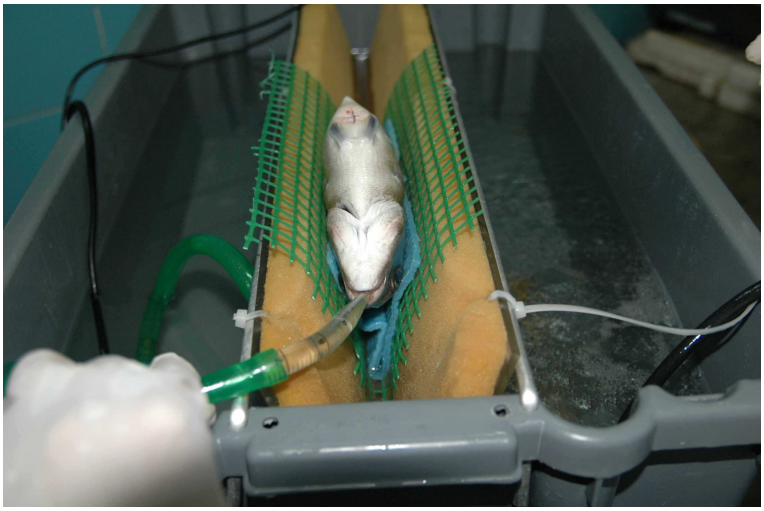

Figure 17 - Surgery bench with V-shaped berth and water pumping directly into the anesthetized fish's mouth.

This berth allowed the fish to remain moist while aerated water was continuously pumped into the mouth of the fish through a tube. This water contains a diluted solution of 2-Phenoxyethanol at 0,2 ml l-1 so that the anesthesia is not removed but it does not progress into deeper anesthesia.

The fish scales between the pelvic girdle and the anus are removed and the area is cleaned with an antiseptic iodine based paste (Betadine). A longitudinal clean incision of around 1cm length is cut at mid distance from the pelvic girdle and the anus (Figure 18).

Figure 18 - Ventral incision in an anesthetized white sea bream, with acoustic tag already inserted, just before suture.

The acoustic tag, previously cleaned with the antiseptic paste is inserted into the body cavity (Figure 19). The incision is then closed with a single suture using a non-absorbable nylon monofilament (Braun Dafilon 3/0 DS19 45 cm). This suture was recommended since it prevents seepage of sea water into the body cavity and it is easier to handle.

Before placing the fish into the recovery tank filled with clean sea water, the fish was measured, weighted and tagged externally with a numbered T-bar anchor tag.

Surgically implanted fish were kept under observation for a week before release at sea. This allowed making sure the incision would heal in clean conditions and prevent the tag from being rejected.

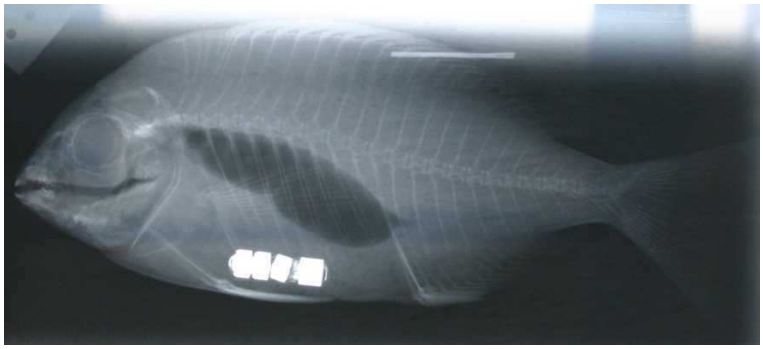

Figure 19 - X-Ray of a white sea bream (*Diplodus sargus*) showing the position of the surgically implanted acoustic tag.

Active telemetry

Active telemetry refers to actively pursuing the tagged fish in order to detect it's presence and movements. This is carried out with a hydrophone connected to a receiver. In this case the receiver was a VR100 model from VEMCO which includes a GPS unit (Figure 20). Each time a fish is positively identified, the GPS position is registered along with the ID of the fish. In addition a measure of the intensity of the sound received is registered.

To quickly locate a fish in a general area an omnidirectional hydrophone is used since it detects at 360 degrees. For fine positioning and getting an accurate position of the fish a directional hydrophone is used since it has a very narrow angle of reception (around 20 degrees at 69KHz). The increase in sound intensity indicates the proximity of the fish.

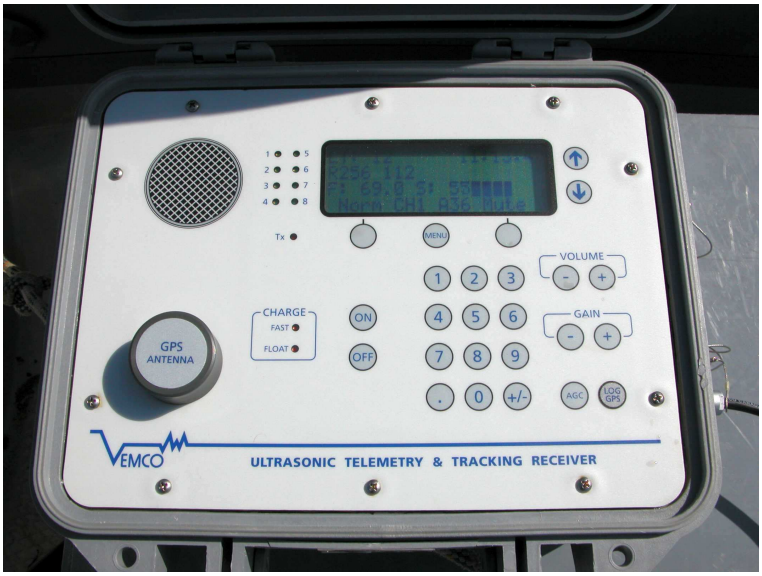

Figure 20 - VR100 acoustic receiver. The display shows that fish with ID 112 was identified. The GPS unit allows association of a geographic position to the fish detection.

In the studies carried out, both the omnidirectional and the directional hydrophones were linked to a frame which was securely fastened to the boat's infrastructure. This arrangement allowed to quickly switch from one hydrophone to the other.

Figure 21 - Hydrophone frame fixed to the boat's infrastructure allowed for quick switching during active telemetry.

Passive telemetry

Passive telemetry consists of using fixed stations forming an array of receivers where data about fish positively identified is registered in the receiver's solid state memory. The major advantage of passive telemetry is that it allows data to be recorded 24hours/7 days a week in the area covered by the receivers. The obvious disadvantage is that data is stored so it is not available in real time and all data could be lost if the device is lost or damaged. Since this equipment is commonly used within areas actively fished it is quite common to register interactions with live fishing gear (Figure 24).

To define the correct configuration of receivers in an array, the study must start with an assessment of the acoustic range at the location of the future study. Several factors can affect acoustic range (e.g. environmental noise, boat traffic) so this must be done for each location even if the equipment is the same.

After determining the acoustic range the experimental design can be planned based on the determined range.

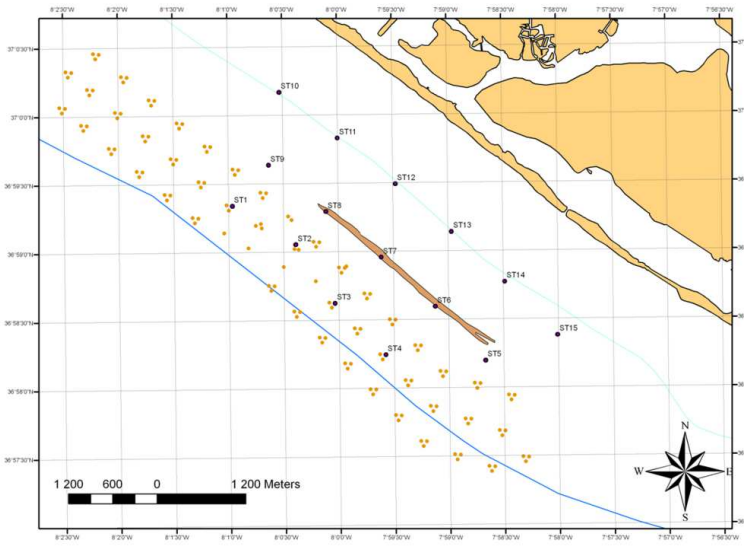

Figure 22 - Example of an array design with receivers deployed in three lines on the inshore sandy bottom, along a natural reef (middle line) and on artificial reefs located in front of the Ria Formosa. The distance between stations is defined by the acoustic range.

Unlike the active telemetry receiver which provides a GPS position, it is the user who defines the GPS position of the passive telemetry station. In the studies carried out two methods were used for positioning the passive receivers: 1) placed on the bottom, with hydrophone turned up, and tucked inside a “docking station” (Figure 23); or 2) floating in mid-water, anchored to the bottom or tied to an artificial reef, with a mid-water float placed above, keeping it upright and with the hydrophone facing down (Figure 24).

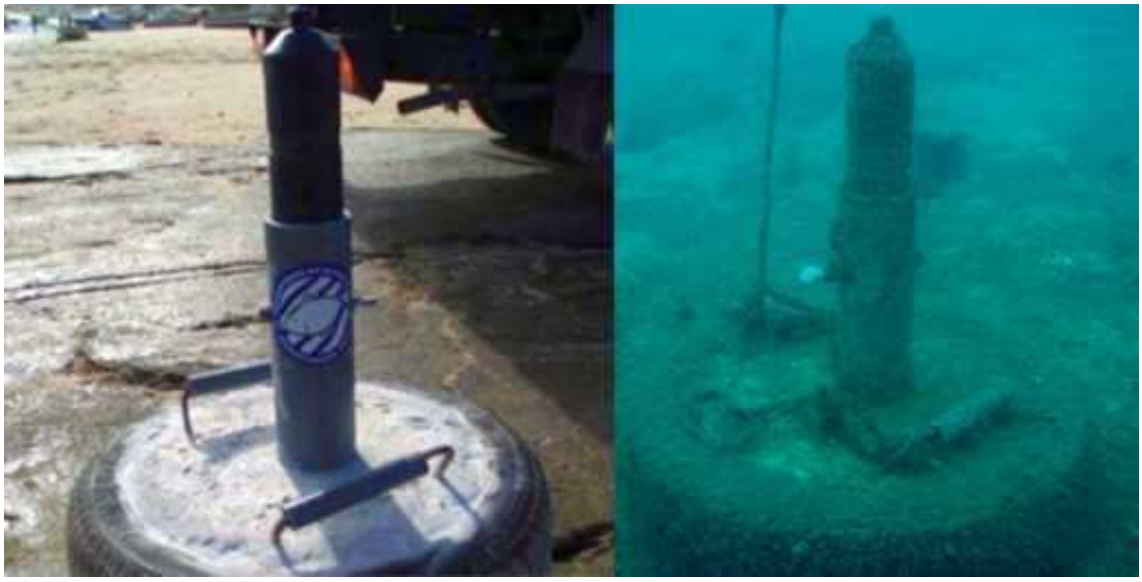

Figure 23 - Acoustic receiver type VR2 in "docking station" before being deployed (left) and underwater for a month (right)

Figure 24 - Acoustic receiver placed in mid-water with part of a snagged monofilament net.

CHAPTER 3

Genetic differences between wild and hatchery populations of *Diplodus sargus* and *D. vulgaris* inferred from RAPD markers: implications for production and restocking programs design.

Authors: Jorge C. Pereira, Pedro G. Lino, Alexandra Leitão, Sandra Joaquim, R. Chaves, Pedro Pousão-Ferreira, Henrique Guedes-Pinto and Miguel N. Santos

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Abstract

Restocking and stock enhancement programs are now recognized as an important tool for the management of fishery resources. It is important, however, to have an adequate knowledge on the genetic population structure of both the released stock and the wild population before carrying out such programs. In this study, random amplified polymorphic DNA (RAPD) markers were applied to assess genetic diversity and population structure of wild and hatchery populations of the white seabream *Diplodus sargus* and the common two-banded seabream *D. vulgaris* (Sparidae). The estimated values for intra-population genetic variation, measured using the percentage of polymorphic loci (%*P*), Shannon index (*H'*), and Nei's gene diversity (*h*), showed high values for all populations. The percentage of genetic variation within *D. sargus* and *D. vulgaris* populations, based on coefficient of gene differentiation, reached 82.5% and 90% of the total genetic variation, respectively. An undeniable decrease in genetic variation was found in both hatchery populations, particularly in *D. sargus*, compared to the wild ones. However, the high values of variation within all populations and the low levels of genetic variation among populations did not indicate inbreeding or depression effects, thus indicating a fairly proper hatchery management. Nevertheless, the results of this study highlight the importance of monitoring the genetic variation of hatchery populations, particularly those to be used in restocking programs. The creation of a genetic baseline database will contribute to a more efficient conservation management and to the design of genetically sustainable restocking programs.

Introduction

Restocking and stock enhancement have been used as tools to recover stocks of commercially overexploited marine fish in several countries (Støttrup and Sparrevohn 2007). However, the massive releases of hatchery-produced fish have raised concerns

on their genetic effects on wild populations at 2 levels: (1) hatchery fish may have a reduced genetic variability (Taniguchi 2004), and this may eventually lower the genetic diversity in the population into which it is released; and (2) genetic viability of wild populations may be eroded by transplantation of non-native fish or their hatchery-derived offspring (Tringali and Bert 1998). It is therefore necessary to have adequate knowledge on the genetic population structure before carrying out any restocking or stock enhancement project (Cross 2000), which can be achieved by molecular genetic analysis (Ward 2006). The white seabream *Diplodus sargus* (Linnaeus 1758) and the common two-banded seabream *D. vulgaris* (Geoffroy Saint-Hilaire 1817) are highly commercially valuable species, naturally occurring in southern Portugal (Algarve coastal waters). These are demersal fish belonging to the family Sparidae, whose geographic distribution extends from the Bay of Biscay to Cape Verde Islands. Their bathymetric range extends from the shallow subtidal zone down to the depth of about 90 m (Whitehead et al. 1986). *Diplodus sargus* inhabits littoral waters on rocky bottoms and sand close to rocks, while *D. vulgaris* also occurs on sandy bottoms (Whitehead et al. 1986). Both species are morphologically very similar, but are easily distinguished based on external features. They have the same trophic level (Guidetti and Sala 2007), their reproduction season overlaps (Gonçalves et al. 2003; Erzini et al. 2001), but they do not tend to form mixed schools during mating (M.N. Santos and P.G. Lino, oral comm.).

The Portuguese Fisheries and Marine Research Laboratory (IPIMAR) produces and rears these 2 species in its own Aquaculture Research Station (EPPO). The capacity to mass produce fish species is one of the conditions to consider a species for restocking (Bell et al. 2006; Støttrup and Sparrevohn 2007). Although presently both stocks do not require such intervention, IPIMAR has been testing the potential of these species for

restocking, since according to Bell et al. (2006), there are no “shortcuts” to identify whether hatchery releases will be a viable management option for each situation.

The establishment of a founder stock for sparid hatchery production should be considered the fundamental step in broodstock management, since it will determine the population’s genetic variability and inbreeding that will take place by future crosses. In general, the effective size of founder populations is conditioned by hatchery techniques constraints, which may result in the use of only a few individuals as broodstock. This practice may lead to erosion of the genetic diversity of the progeny stocks (Alarcón et al. 2004). Therefore, proper hatchery management and breeding programs should be implemented, both for genetic enhancement programs and for the design of restocking or stock enhancement strategies. In order to carry out such programs, information on the genetic background of hatchery populations and on the genetic relationships between hatchery and the wild populations of these species are fundamental.

The use of molecular markers applied to stock assessment has often cast light on population sub-structure, and given useful information for the management of fishery resources (Allendorf and Phelps 1980). RAPD (random amplified polymorphic DNA) is a polymorphic assay based on the amplification of random DNA sequences, using primers with arbitrary nucleotide sequences.

RAPD is a low-cost, simple technique, which requires no previous sequence information and in which a large number or putative loci may be screened (Weising et al. 1995). However, this technique has some disadvantages, associated mainly with dominance, reproducibility, homology inferences, and artifact fragments (Jones et al. 1997; Harris 1999; Ali et al. 2004).

The RAPD method (Williams et al. 1990) has been widely used in molecular biology laboratories and frequently applied to reveal population-genetic variation, divergence,

and biogeography (Schaal and Leverich 2001). In aquaculture fish species, it has already been successfully applied to catfish (Liu et al. 1998), discus (Khol et al. 1999), red seabream (Jiang et al. 2004), carp (Wang and Li 2004), gilthead seabream (Bilgen et al. 2007), and flounder (Liu et al. 2007).

The main objective of this study was therefore to define the genetic structure of both hatchery-produced and wild populations of *D. sargus* and *D. vulgaris* from Southern Portugal, in order to estimate the degree of potential genetic erosion of hatchery populations, by comparing their genetic variability with that of geographically close wild stocks.

Materials and methods

Sample collection and DNA extraction

For each species, *D. sargus* and *D. vulgaris*, 20 hatchery-produced fish were obtained from the IPIMAR's EPPO, while 20 wild fish were obtained from natural southern Portuguese populations. All wild fish were captured by the local fishing fleet, at the same area on the south Algarve coastal waters (southern Portugal). Fin clips were cut immediately after collection of the individuals, placed in 95% ethanol, and stored until further processing. The protocol used for genomic DNA extraction is based on the use of the automatic system equipment QuickGene-810 and an adaptation of the QuickGene DNA Tissue kit developed by FUJIFILM LIFE SCIENCE. The fin clips (5–25 mg) were cut in small peaces and placed in a 2-mL Eppendorf tube. 180 µL of MDT (Tissue Lysis Buffer) and 20 µL of EDT (Proteinase K) were added to the mixture and incubated overnight at 55ºC. The subsequent addition of 180 µL of LDT (Lysis Buffer) was followed by vortexing for 15 s, and a flash spin down. After incubation at 70ºC for 10 min, 240 µL of absolute ethanol was added, and the tubes were vortexed and spun down. Finally, the lysate was transferred to a cartridge of the automatic nucleic-acid isolation system QuickGene-800, and the "DNA tissue mode" was selected.

RAPD amplification

A series of optimization experiments were conducted following the protocol of Williams et al. (1990), with various concentrations and purity of template DNA, dNTPs, MgCl 2 concentration, and Taq polymerase, to determine which conditions produced

the strongest and most reproducible patterns. A total number of 20 RAPD primers (Operon Technologies) were screened. Among them, 6 RAPD primers (Table 1) produced clear and reproducible bands, so they were selected for amplification of all the *D. sargus* and *D. vulgaris* DNA samples. To test the reproducibility of the bands, 3 replicates were analyzed for all selected primers in which contamination controls were added. The amplification reactions were performed in volumes of 25 µL, containing 50 ng of genomic template DNA, 2 mM MgCl 2 , 100 µM of dATP, dCTP, dGTP and dTTP each, 0.2 µM of the primer, and 0.5 units of Taq DNA polymerase (Fermentas, Life Sciences). Amplification was performed in a Thermal Cycler (T-personal, Biometra) in a total of 45 cycles: 1 min at 94ºC, 1 min at 30–36ºC, and 2 min at 72ºC, using the fastest possible transitions between each temperature. The total volume of the PCR products were evaluated in 2% agarose gels and visualized by ethidium bromide staining. After electrophoresis, DNA bands profiling were observed under UV light, and the images were saved in a gel analyzer (UVIDOC).

Data analysis

Amplified fragments were scored as binary data, i.e. presence as 1 and absence as 0, for homologous bands. Only data generated from reproducible bands were used for statistical analysis. The number of polymorphic loci, percentage of polymorphic loci (%P), observed number of alleles (n_a), effective number of alleles (n_e), Nei's gene diversity (*h*), and Shannon index (*H'*) were estimated using the program GenAlEx 6.1 (Peakall and Smouse 2007). Nei's (1987) coefficient of gene differentiation (G_{ST} , analogous to the fixation index F_{ST}) and gene flow (N_m) were estimated using POPGENE program version 1.32 (Yeh et al. 1997). To calculate the percentage of

polymorphic loci with the most common allele not exceeding 95% ($\%P_{95}$) and Nei's (1972) genetic distance, we used TFPGA 1.3 software (Miller 1997). To estimate the genetic structure of *D. sargus* and *D. vulgaris* populations, a measure of genetic differentiation (Φ_{PT}) was estimated using the non-parametric analysis of molecular variance (AMOVA) with the program GenAlEx 6.1 (Peakall and Smouse 2007) for variation among individuals within populations and among populations. Allele frequency was calculated according to Lynch and Milling (1994).

Results

Genetic diversity within populations

The 6 selected primers amplified 161 and 168 clear and reproducible bands, for *D. sargus* and *D. vulgaris* populations, respectively (Table 1). The size of the major amplified bands ranged between 200 and 1500 bp.

Intrapopulation genetic diversity values, estimated by the Shannon index (*H'*), Nei's gene diversity (1973) (*h*), number of observed and effective alleles (n_a and n_e), and percentage of polymorphic loci (%P and $\%P_{95}$), are presented in Table 2. A high percentage of polymorphic loci (%P) was observed for both species. However, values for hatchery populations were lower than those for wild populations. The *H'* and *h* values were higher in the wild than in the hatchery populations for both species. Nevertheless, the results demonstrate that the intrapopulation genetic variation was high within populations in all 4 cases.

Genetic variation between populations

Analysis of molecular variance (AMOVA) and coefficient of gene differentiation (G_{ST}) (Nei 1987; Excoffier et al. 2005), calculated with the programs mentioned earlier, allowed us to examine the hierarchical partitioning of genetic variation in the populations of *D. sargus* and *D. vulgaris*. According to the values obtained with the application of multi-population analysis (Nei 1987) (Table 2), the total genetic diversity (H_T) was 0.2787 for *D. sargus* and 0.2305 for *D. vulgaris*, whilst the values of the coefficient of gene differentiation (G_{ST}) were 0.1755 (*D. sargus*) and 0.1008 (*D. vulgaris*). This means that 82.5% and 90% of the total genetic variation is within *D. sargus* and *D. vulgaris* populations, respectively. The *D. sargus* and *D. vulgaris* populations presented high values of gene flow (N_m= 2.3489 and 4.4612, respectively), which is consistent with previous findings.

The AMOVA results indicated that within-population variation accounted for 77% and 88% of the total genetic variation, whilst between-population variation accounted for the remaining 23% and 12%, for *D. sargus* and *D. vulgaris*, respectively. The analogue of fixation index (Φ_{PT}), calculated with AMOVA, reached 0.225 and 0.122 for *D. sargus* and *D. vulgaris*, respectively, suggesting moderate to low genetic differentiation between the wild and hatchery populations of both *Diplodus* species. The analyses of these values lead us to a conclusion that there are no major genetic differences between the wild and hatchery populations in both studied species.

Discussion

Avoiding extinction of heavily exploited populations is the first goal of any nature conservation plan (Man et al. 1995), but since all environments ultimately change and will probably change at an ever-increasing rate due to human influence, then conservation programs must also maintain the capacity of fish to adapt genetically, preserving genetic variability. Therefore one of the major concerns should be the maintenance of the existing genetic variation, both within and among different populations, maintaining high levels of heterozygosis and preserving allele richness (Meffe 1986).

Several methods based on PCR have been developed for genetic analysis of several fish species. RAPD fingerprinting revealed to be less time consuming and less expensive (Liu and Cordes 2004; Liu et al. 2004). The disadvantage of its low reproducibility can be overcome by replicating exactly the same laboratory conditions and assaying 2 or more times to ensure reproducibility. Consequently, RAPD is one of the best methods for the assessment of genetic variation among populations in species where little molecular genetic information is available. This technique has been previously successfully applied to population analysis of several fish species (Liu et al. 1998; Jiang et al. 2004; Wang and Li 2004), including specific comparisons between wild and hatchery populations of the same species (Khol et al. 1999; Bilgen et al. 2007; Liu et al. 2007).

Genetic diversity within populations is highly important for the adaptation to changing environments and, as a consequence, for long-term survival of a species. In this work, all studied populations of *D. sargus* and *D. vulgaris* revealed high within-population

variation, estimated by several genetic parameters. Lenfant and Planes (1996), González-Wangüemert et al. (2004) and Domingues et al. (2007), had also previously found high values of within-population genetic diversity in several *D. sargus* populations. However, as far as *D. vulgaris* is concerned, to our best knowledge, this is the first genetic population study performed.

The analysis of the genetic structure in both species also revealed that the level of between-population variation was higher in *D. sargus* than in *D. vulgaris*. AMOVA results showed that genetic variation was higher within populations than between populations, for both *Diplodus* species. The obtained low to moderate genetic differentiation between populations, associated with the reduced values of genetic distance and high values of gene flow, allows us to state that these populations correspond to a genetic structure of a single population by species. Similarly low level of between-population variation was found by D'Anna and Badalamenti (2000) between hatchery and wild *D. sargus* populations from Golfo di Castellammare (Sicily).

In conclusion, results of this study give a preliminary view of genetic variation both within and between these populations. Both hatchery populations analyzed, but particularly that of *D. sargus* show an unquestionable decrease in genetic variation in comparison to the wild ones. However, the high values of variation within populations and the low levels of variation among populations did not reveal any inbreeding or depression effects, thus indicating a fairly proper hatchery management. The IPIMAR hatchery, being a research facility, is particularly careful with this issue, having a 20% annual replacement of the broodstock with new wild specimens. Nevertheless, an assessment of the genetic structure of hatchery populations should be conducted periodically along the selective breeding programs, in order to avoid any major reduction in genetic diversity of hatchery populations, particularly those to be used in

future restocking programs. The intrapopulation genetic variation is, in fact, an essential parameter of species adaptation to environmental changes. When a gene pool from a population narrows and loses genetic plasticity, it becomes more prone to changes in environmental conditions and therefore is more susceptible to extinction (Guttman and Berg, 1998). The creation of genetic baseline data with the use of several other molecular markers will certainly contribute to a more efficient conservation management and should be used for each species prior to any massive release that might affect the wild population.

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Table 1. Summary of used RAPD primers and band data of the studied *Diplodus* species. T_a= annealing temperature; N = total number of bands; %P = percentage of polymorphic bands

Name	Primers		*D.sargus*			*D. vulgaris*		
	sequence (5′→ 3′)	T_a (°C)	N	%*P*		N	%*P*	
				hatchery	wild		hatchery	wild
OPE03	CCAGATGCAC	36	35	71.5	100	33	69.6	81.8
OPE05	TCAGGGAGGT	33	22	59.0	90.9	21	61.9	76.1
OPE10	CACCAGGTGA	36	15	86.6	86.6	27	70.5	88.9
OPE12	TTATCGCCCC	33	30	53.0	80.0	31	45.1	96.7
OPE14	TGCGGCTGAG	33	37	48.0	93.3	32	65.6	71.8
OPE15	ACGCACAACC	33	22	31.8	63.6	24	75.0	79.2

Table 2. Summary of genetic variation statistics for the studied *Diplodus* species

	D. sargus		*D. vulgaris*	
	hatchery	wild	hatchery	wild
Percentage of polymorphic loci (%*P*)	56.5	87.6	64.2	83.0
Percentage of polymorphic loci* ($\%P_{95}$)	49	68	52%	68%
Observed number of alleles (*na*)	1.5652	1.8758	1.6429	1.8274
Effective number of alleles (*ne*)	1.3318	1.4300	1.3140	1.3661
Nei's (1973) gene diversity (*h*)	0.1931	0.2664	0.1919	0.2317
Shannon index (*H*)	0.2900	0.4120	0.2967	0.3645
Gene flow (N_m)	2.3489		4.4612	
Total gene diversity (H_T)	0.2787		0.2355	
Coefficient of gene differentiation (G_{ST})	0.1755		0.1008	
Nei's (1972) genetic distance	0.1187		0.0600	

*with the most common allele not exceeding 95%

CHAPTER 4

Preliminary results of hatchery-reared seabreams release at artificial reefs off the Algarve coast (southern Portugal): a pilot study.

Authors: Miguel N. Santos, Pedro G. Lino, Pedro Pousão-Ferreira and Carlos C. Monteiro

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Abstract

In 2001 a pilot project of fish restocking began using reared juveniles of two native species: the white seabream (*Diplodus sargus* Linnaeus, 1758) and the gilthead seabream (*Sparus aurata* Linnaeus, 1758). Between 2001 and 2004 more than 13,600 juveniles of different sizes (over 7,500 white seabreams and 6,100 gilthead seabreams) were tagged (FLOY T-Bar anchor FD94) and released on the artificial reefs (ARs) areas, aiming to evaluate the efficiency of restocking. The preliminary results of this ongoing study based on caught fish show that the number of days at liberty ranged from 1 to 340, while the distance traveled ranged from 0 to 67 nm. However, the mean dispersal distance was less than 11 nm from the release location. A behavioral deficit of the reared seabreams in the use of refuges and feeding was observed during the first week after release. However, thereafter the gut content analysis suggested that the reared specimen were able to search for food and feed on the available preys. These results suggest that restocking associated with ARs may be used as an additional tool within an integrated coastal management plan, aiming at the enhancement of locally important artisanal fisheries.

Introduction

Numerous attempts are underway worldwide to augment the natural supply of fish by various means, ranging from aquaculture to various fisheries-enhancement systems (Munro and Bell, 1997). According to Leber et al. (2004) in recent years, 33 developing countries have reported the stocking of 59 marine or coastal species. Nonetheless, most of the hatchery-based programs for fisheries enhancement have failed (Bohnsack, 1996), the exception being the Japanese experiments with red seabream (*Pagrus major* Temminck and Schlegel, 1843) and Japanese flounder (*Paralichthys olivaceus* Temminck and Schlegel, 1846) (Fushimi, 2001). However, these releases occurred in limited habitats such as coastal lagoons, fjords, estuaries, etc (McEachron et al., 1995).

The causes of such failures have been reviewed by D'Anna et al. (2004) and attributed to a wide range of issues.

The gilthead (*Sparus aurata* Linnaeus, 1758) and white (*Diplodus sargus* Linnaeus, 1758) seabreams are two commercially important species in southern European countries, where catches have declined in the last two decades (FAO, 2004). In the Algarve coast (Southern Portugal) the landings of the white seabream decreased from 200.3 tons to 75.2 between 1987 and 2004, while the gilthead seabream has shown some stability, with mean annual landings of 72 tons (data source: National Fisheries Database). These species are mainly targeted by small-scale and recreational fisheries. Aspects of their biology and ecology are well known (Arias, 1980; Rosecchi, 1985; Gordoa and Moli, 1997; Vigliola and Harmelin-Vivien, 2001). These species are also successfully bred and reared up to the age of about 1 year. However, in the case of *D. sargus* growth rates are slower, making the rearing process inappropriate for intensive aquaculture (Abellan et al., 1994) and thus economically unprofitable.

The present study, carried out in the Algarve, is the first aiming at the enhancement of local marine fisheries by means of restocking. Presently, the enhancement of local fisheries has been mostly based on a program of artificial reef deployment, which started in 1990 (Santos and Monteiro, 1997; 1998). Currently, the Algarve artificial reef complex consist of seven large sized systems, which cover a total area of 43.5 km^2, making use of more than 20,500 concrete blocks with a total volume of over 100,000 m^3 (Santos and Monteiro, 2001). The white seabream is a common species in these ARs (Santos and Gaspar, 2002), while the gilthead seabream is rare, although being a common species in the neighboring sandy areas.

This article reports preliminary results of release experiments using two species of hatchery-reared seabreams, aiming at evaluating their potential usefulness within an integrated coastal management plan for enhancing locally important artisanal fisheries.

In particular, the objectives are: (i) to evaluate the ability of hatchery-reared young seabreams to adapt to the wild and (ii) to evaluate fish dispersion after release at the artificial reefs.

Material and methods

STUDY SITE

The artificial reef systems of Olhão and Faro/Ancão are located off the Ria Formosa (Algarve, south Portugal), a highly productive ecosystem that acts as a nursery, supplying the most important fish stocks of the coastal waters (Monteiro et al., 1987, 1990), while the Vilamoura artificial reef system is located slightly to the west (see Figure 1). The artificial reef systems were deployed between 1990 and 2004, 2.5 to 4.8 km off-shore, on flat sandy or sandy/muddy bottoms. A few scattered patches of bedrock were recorded on the bottom of the Vilamoura and Faro/Ancão areas. Each artificial reef system consists of between 7 and 52 assemblages of 35 concrete cubic units (2.7 m^3 each) and between 5 and 18 groups of 4 large concrete structures (174 m^3 each) (for details see Santos and Monteiro, 2001).

TAG AND RELEASE

The released specimens were hatched and reared at IPIMAR's aquaculture facility in Olhão, starting from a wild parent stock caught in the area. The seabreams were tagged using dart style tags (T-anchor Bar FD-94 and FF-94, from ''Floy Tag''), following the procedure suggested by Parker et al., 1990. Between November 2001 and July 2004, 14 batches of fish of different sizes were tagged and released (7,520 white seabreams and 6,102 gilthead seabreams). The percentage of mortality due to tagging and/or handling was negligible (< 0.5%). Data and statistics on the fish released are summarized in Table 1.

The release was done using procedures to minimize stress on the fish. Fish release occurred at the Olhão, Faro or Vilamoura artificial reef systems at one of the reef sets (assemblages of 35 concrete cubic units, see Figure 1)

DATA COLLECTION

Fish returns data analyzed in this article were recorded for almost 3 years (from November 2001 until September 2004). Visual censuses, which allowed the estimation of fish density (no. fish/m^3 water), were carried out by a SCUBA diver at the Faro/Ancão artificial reef system, for batches of *D. sargus* released in September 2002 and May 2003. The visual censuses extended for a 3 month period, at different time intervals (1, 3, 5, 8, 15 and approximately 30, 45, 60 and 90 days after release). Three artificial reef sets were sampled each time. Overall, a total of 81 fish counts were made using the stationary point count method developed by Bohnsack and Bannerot (1986).

Underwater photography and video recording, together with observations made during visual census, allowed qualitative information on the spatial distribution and on the behavior of released seabreams on the artificial reefs to be gathered.

Returns from recreational and professional fishermen were used to estimate fish dispersion. Information requested from the fishermen included: date of capture, location, fishing gear used, fish size and weight. Fish returned by fishermen were measured by scientific staff and when the fishermen did not want the fish for their own consumption, an analysis of the digestive system was carried out. Items present in the digestive system (including stomach and intestine) of *D. sargus* were analyzed and identified to the lowest possible level.

DATA ANALYSIS

Exponential models were fitted to the mean abundance over time using:

$$Abundance(N_t) = a_n e^{-b_n t}$$

where, Abundance(Nt) is the density of fish in number over time, a_n is the intercept, b_n is the parameters defining the rate of decrease, and t is time after release (number of days). In order to compare the results from the summer and spring experiments in terms of fish abundance, data were log transformed [log(1+x)] and the Student's t test was used for comparing the slopes (Zar, 1996).

Results

Visual census on artificial reefs

A total of 1456 white seabreams were counted on the Faro/Ancão artificial reef system over the two periods. The highest abundances were registered immediately after release, decreasing rapidly within the first week. The last *D. sargus* were observed 30 days after the release in both experiments. The estimated slops of the models for the two experiments were not significantly different ($t=0.0006$, $p<0.01$), and thus a curve was fitted to the pooled data (Figure 2). There was a rapid decrease in abundance of *D. sargus* over time.

During the visual census, namely within the first three days after release, divers observed a behavioral deficit of the white seabream in the use of the artificial reef refuges. The fish formed shoals, swimming around the modules as they usually do while in the rearing tanks and showed no attempt to hide within the modules in the presence of divers or natural predators, such as large sea bass (*Dicentrarchus labrax* Linnaeus, 1758) or European conger eel (*Conger conger* Linnaeus, 1758). Also, during the first week the fish did not forage on the modules as wild fish usually do. However, after about 10 days, small groups of two or three tagged fish were observed, together with other species of the same Genus (*Diplodus annularis* Linnaeus, 1758; *D. bellottii*

Steindachner, 1882; and *D. vulgaris* Geoffroy Saint-Hilaire, 1817) moving, foraging and hiding among the artificial reefs modules.

Digestive system contents

A total of 17 *D. sargus* specimens were returned by fishermen, which allowed the analysis of their digestive system contents. Fish caught within the first 8 days after release (8 specimens, with a mean size of 19.6 cm) showed no contents in their digestive system. After 11 days after release all the specimens (n=9, mean size of 19.9 cm) had items in their digestive system.

The prey items included algae, bryozoans, gastropods, crustaceans and fish remains. Among these the most frequent items were the crustaceans, namely crabs belonging to Brachyura Order.

Fish returns

Of the 13622 released fish, 337 *D. sargus* and 369 *S. aurata* were returned, for an overall catch rate of 5.2%. The percentage of returns per batch ranged from 0.2% to 8.6% and 2.8% to 11.2%, for the white and gilthead seabreams, respectively. The overall return rate for the white seabream was 4.5%, while for the gilthead seabream it was 6% (Table 1). The maximum days at liberty observed, were 287 and 340 for the gilthead and white seabream, respectively. In terms of maximum dispersal range, both species showed similar values, although *D. sargus* reached the maximum distance in much less time. On the other hand, this species showed lower catch rates closer to the ARs and a higher mean dispersal distance than the gilthead seabream (see Table 1 for details).

Discussion

Our observations highlight the fact that during the first days after release the fish show a group behavior. This behavior of reared species has been previously reported by Kudoh et al. (1999) for the red seabream (*Pagrus major*) in Japan and by D'Anna et al. (2004) for *D. sargus* in Sicily. The latter authors also reported that the white seabream do not flee into reef holes or crevices, forage, and are not are afraid of potential predators. The same reaction was also reported to us by spear fishermen who observed our tagged specimens (L. Sousa and F. Reis, pers. comm.). As suggested by D'Anna et al. (2004), this behavioral deficit is probably linked to the long time in captivity, with no chance to experience different habitats. Moreover, the fact that during the first days we observed them swimming in large groups could be also a consequence of their lack of natural behavior or, as mentioned by Macpherson (1998), the gregarious habit of the juveniles. These results support the opinion of many authors, who believe that reared specimens once released in the open sea, are not able to perceive environmental *stimuli* useful for their settlement and they are not able to exploit available food resources (Olla et al., 1994). Thus, as mentioned by D'Anna et al. (2004) it is clear that such behaviors might have negative effects on the survival of released individuals. However, this might not necessarily compromise the success of the restocking experiments. In fact, our *in situ* observations and findings regarding the digestive system items, confirmed that only 11 days after release all fish (which were larger than 19.5 cm) had ingested some food items. Thus, farmed individuals do search for food and hunt for live prey, at least during the initial stages after their release. The low number of fish analyzed does not allow for robust conclusions, but it was interesting to notice that the primary prey found is Brachyura, i.e. crabs which are not used as bait, and were found in the digestive system since day 11. This means that *D. sargus* reared in captivity has the instinct and ability to

feed on hard shelled active organisms which are commonly found in the diet of wild white seabreams (F. Leitão, IPIMAR, unpublished data).

Wild specimens of *D. sargus* have constantly been observed on the Algarve ARs, while the occurrence of *S. aurata* on these structures is occasional. However, the tagged seabreams left the reef rather early to move towards shallower coastal waters. The same behavior was previously reported by D'Anna et al. (2004) for *D. sargus* tagged and release on ARs in the Gulf of Castellammare and Sánchez-Lamadrid (2002) for *S. aurata* tagged and released in the Gulf of Cadiz (SE Spain). This behavior is most likely to be due to some ecological factors, rather than due to the type of substratum or to other features of the artificial habitats. Ongoing studies on the contribution of the Algarve ARs to the diet of *D. sargus* (F. Leitão, IPIMAR, unpublished data); suggest that at least food and shelter are not limiting factors for this species. As suggested by D'Anna et al. (2004) such preference by *D. sargus* for shallower waters could be due to a search for adjacent shallower artificial habitats such as breakwaters and harbors, which are particularly suitable for the settlement and growth of juveniles and pre-adults fishes. In fact, a considerable portion of the captured white seabreams occurred in such areas, where the availability of numerous holes and crevices of different dimensions, in shallow and sheltered waters, seem to be an important factor for the fish settlement. These findings are also supported by some studies carried out on wild white seabream (Biagi et al., 1998; Macpherson, 1998). These authors reported specific habitats associated to the early stages of the life cycle of *D. sargus*.

Regarding the gilthead seabream the situation is slightly different since most fish (more than three quarters of fish released) were captured within 10 nautical miles of the release site. This shows that although gilthead seabream also move to shallower waters, they tend to disperse less than the white seabream. This could be related to the location of feeding grounds, namely of juvenile bivalves beds, which occur near the coast at less

than 10m deep (M.B. Gaspar, IPIMAR, pers. comm.). This hypothesis is based on the information that the primary preys in the diet of wild *Sparus aurata* are mollusks and in particular bivalves (Arias, 1980; Rosecchi, 1985). This behavior suggests that this species has a low reef dependence, which is reinforced by the fact that during our regular underwater observations (M.N. Santos, IPIMAR, unpublished data) we do not observe regularly wild gilthead seabreams in the artificial reefs.

The preliminary results of this study seem to indicate that hatchery-reared young white seabreams, although showing an initial behavior deficit regarding predators, have the instinct to feed on live preys after a short period. This appears to be a good indicator that they can adapt to the wild successfully. On the other hand, specimens of both species released at the artificial reefs do not stay inside the artificial structures for a long period, but their dispersal is mostly limited to neighboring areas (less than 11 nm). These results suggest that these species might be adequate for restocking at the regional level and that fish restocking may be used as an additional tool within an integrated management plan for local fisheries enhancement. However, these results should be regarded as preliminary, since several important aspects such as growth, mortality and habitat selection must be further investigated.

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AUTHOR(S) ADDRESSES

IPIMAR – CRIPSul, Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal

Corresponding author e-mail: <*mnsantos@cripsul.ipimar.pt*>

Table 1 - Summarized characteristics of the 14 batches of fish released and respective return data. SD = standard deviation, nmi = nautical miles, and FL = fork length.

	Sparus aurata	***Diplodus sargus***
Total number of fish released	6102	7520
Number of batches	7	7
Minimum fish size (Fork length, cm)	10.5	11.6
Mean fish size ± SD (Fork length, cm)	19.0 ± 2.8	16.5 ± 2.6
Maximum fish size (Fork length, cm)	34.5	23.3
Minimum fish weight (g)	24	34
Mean fish weight ± SD (g)	170.9 ± 92.4	151.2 ± 64.5
Maximum weight (g)	1006	416
Total weight of released fish (kg)	1014.9	1136.5
Total number of fish returned	378	337
Total percentage of returns	6.2%	4.5%
Maximum days at sea	287	340
Minimum dispersal distance nmi (days)	0 (163)	0.5 (49)
Mean dispersal distance ± SD nmi	6.3 ± 8.4	10.4 ± 9.1
Maximum dispersal distance nmi (days)	65 (199)	67 (42)
Percentage fish captured at < 5 nmi	65.1%	27.1%
Percentage fish captured at < 10 nmi	77.2%	46.8%
Percentage fish captured at < 20 nmi	90.6%	80.6%

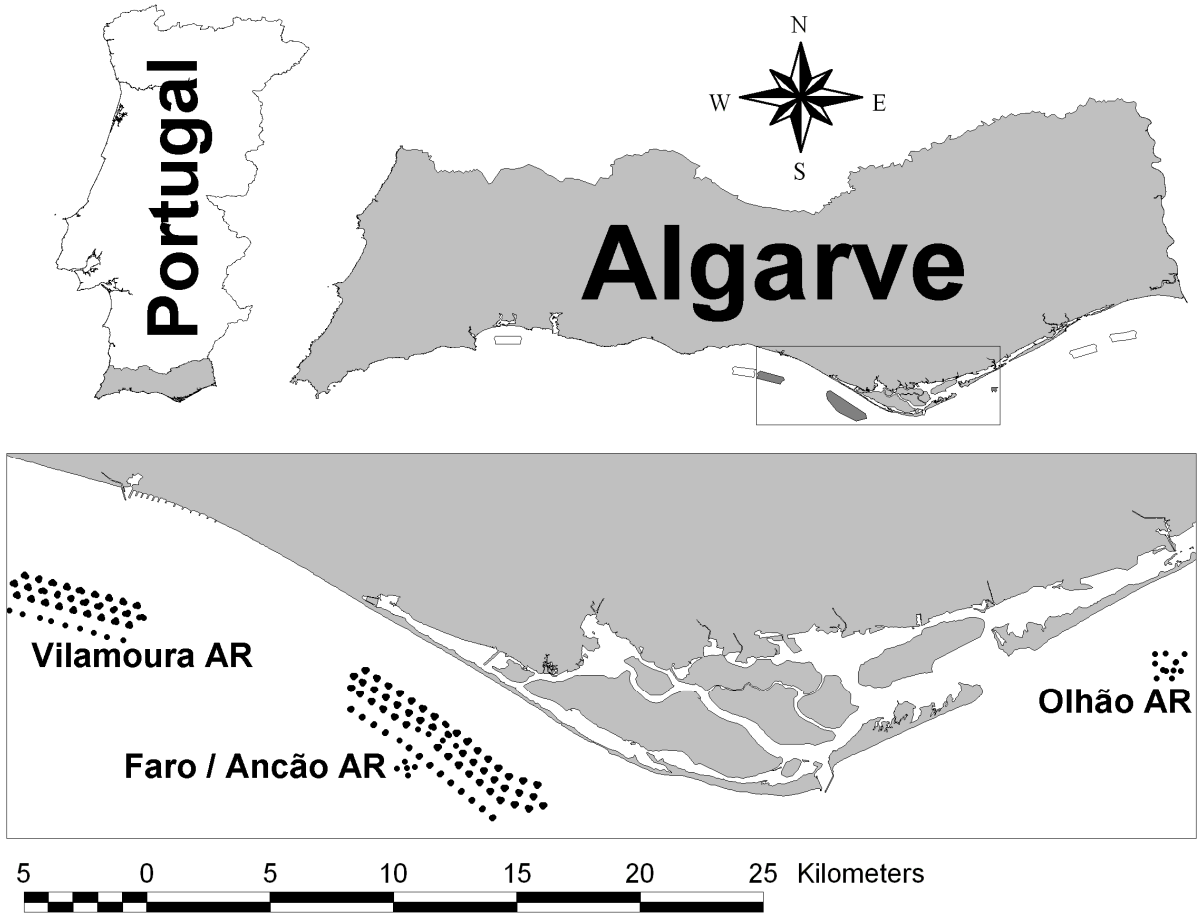

Figure 1 - Geographical location of the Algarve coast (Southern Portugal), with particular emphasis on the study sites (dark grey areas in box) and the artificial reefs (ARs).

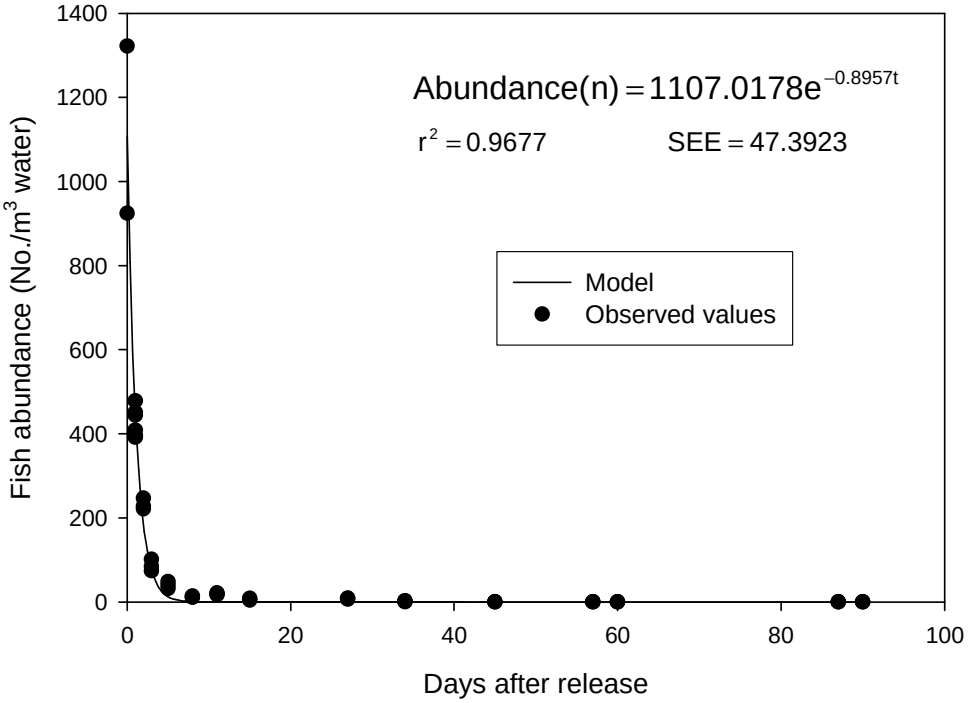

Figure 2 - Progression of the density of the hatched-reared *Diplodus sargus* released at the artificial reefs (points) and fitted model (line). Nt is the density of specimens (No. fish m^{-3}) and SEE is the standard error of the estimated curve.

CHAPTER 5

Diplodus cervinus a new species in aquaculture: is it suitable for restocking? Results of a pilot study in Southern Portugal.

Authors: Lino, P.G.; Santos, M.N and Pousão-Ferreira, P.

Status: To be submitted

Abstract

Tag and release experiments using conventional T-anchor tags were carried out to assess the viability of using the zebra sea bream *Diplodus cervinus* in stock enhancement programs. As a cultured species, the zebra sea bream is a fast grower and has reduced anomalies. The results from the spatial dispersion and weight evolution after release show that the hatchery-reared juveniles of this species have a fast adaptation to the wild once released. Given the commercial value and interest from the sports fisherman, this species appears to be a good candidate for stock enhancement programs off the South coast of the Algarve.

Introduction

As demand rises for seafood associated both with increasing world population and by an increased concern about healthy food among other factors, there is a need to increase supply which fishing from wild resources can no longer sustain (FAO, 2010). One solution found by fisheries managers to increase local stocks from many countries has been the mass release of aquaculture produced fish with the aim either to supplement the current fishable biomass (stock enhancement) or to increase the spawning biomass in the future (restocking) (Bell et al, 2008). Stock enhancement has been successfully used to restore depleted stocks in many areas of the world, especially in Japan (Kitada and Kishino, 2006). According to Støttrup and Sparrevohn (2007) the potential for stocking of a given species is derived from several factors, including the capacity to produce fish in sufficient quantities. The successful production of gilthead seabream

(*Sparus aurata* Linnaeus, 1758) in aquaculture has created the methodology to produce other Sparids in southern Europe, offering a wider variety of species for human consumption (Alarcón and Carmen-Alvarez, 1999), but also opportunities for releasing cultured juveniles into the wild with the aim of increasing fishery recruited populations and catches (Bell et al., 2006). Since 2001, the Portuguese Fisheries and Marine Research Laboratory (IPIMAR) has been carrying out restocking trials with fish produced and reared at the IPIMAR Aquaculture Research Center (EPPO) in Olhão, as it has managed to achieve mass production and rearing of several Sparidae species, namely *Diplodus sargus, D. cervinus, D. vulgaris* and *D. puntazzo*.

The zebra sea bream (*Diplodus cervinus* Lowe, 1838) is a commercially valuable species in southern European countries, where catches have declined in the last two decades (FAO, 2010). In the Algarve coast (southern Portugal) the landings of the zebra seabream have shown some stability, with mean annual landings of less than 2 t (data source: National Fisheries database). However, the commercial value has nearly doubled in less than 10 years, showing that this is an interesting candidate for aquaculture diversification (Figure 1). This is a common species in the Eastern Atlantic Ocean and Mediterranean Sea (Whitehead et al. 1984), living in small pods and having a selective preference for amphipods and polychaetes (Lechanteur and Griffiths, 2003). This species is mainly targeted by small-scale and recreational fisheries. Although there are a few studies on the biology of *D. cervinus* from the Canary Islands (Pajuelo et al, 2003a and 2003b; Dominguez-Seoane, 2005) and from South Africa (Christensen, 1978; Lechanteur and Griffiths, 2003; Mann and Buxton, 1992), to the authors knowledge there are no biological studies on this species in continental European waters.

From a restocking point of view it is important that adaptation to the wild is done on a per species basis (Bell et al, 2006) It is therefore important to increase the knowledge on the species behavior which can contribute to increase their survival, growth and reproduction (Huntingford, 2004). To the authors' knowledge there are currently no scientific published papers on *D. cervinus* comparative growth efficiency in aquaculture or on stock enhancement trials using this species.

The main objectives of this study were: 1) to compare the *D. cervinus* growth efficiency under aquaculture regime with that of gilthead seabream *S. aurata*; 2) to investigate the species ability to adapt to the wild and the potential for stock enhancement based on hatchery produced and reared fish.

Material and methods

Aquaculture production

The zebra seabreams used were hatched and reared at the EPPO. The broodstock originated from the coastal area of the Algarve, South of Portugal, and was kept at a density of 0.6 kg/m^3 (1004.7±768 g/fish, n=6). The larval zebra seabreams were reared in intensive systems and fed on rotifers (3-25 Days After Hatching = DAH), Artemia *nauplii* (10-40DAH) and inert food (after 25 DAH). They were weaned onto dry feed (seabream commercial pellets) after metamorphosis.

Three replicate tanks of 1500 liters were sampled for both *S. aurata* and *D. cervinus* at 0, 2, 10, 20 and 30 DAH. Twenty larvae were measured from each tank under a dissection microscope.

Tagging and releasing

For the restocking experiments juvenile fish were tagged using conventional Floy-Tag brand numbered T-bar anchor tags model FD-94. Fish were anesthetized using Phenoxyethanol in a concentration of 0.2 ppt. When disequilibrium was attained, fish were measured (Fork and Total length to the nearest 1 mm) and weighed (Total Weight to the nearest 0.1 gram).

The fish were collected from the rearing facilities and transported in a fish-transport truck. Transportation time varied from 1 to 3 h and the fish were provided pure oxygen. The oxygen levels were kept at around 80–100% throughout the transport. Water was constantly renewed through the boat's pumping system. This improves water oxygenation and ensures that at release time the water temperature in the holding tanks is the same as the release location.

In 2004 a total of 2201 fish (Table 1) were released by scuba divers near an artificial reef located at 20m depth, during the summer and again in the autumn (Figure 2). In 2005 another batch of smaller fish was released during the summer at a lower depth near a breakwater (2981 fish) and two batches (a total of 2825 fish) were released in consecutive days in the autumn, half at low depth near the same breakwater and half near a natural reef at 20m depth. Because of the weather conditions both of the latter batches were released at the surface using a dip net. Date, numbers, average fish size and weight are given in Table 1.

Posters were disseminated in markets, ports and points of interest to enhance tagged fish catch reporting. The local fishermen were encouraged to return tags and provide data on the catch and the size and condition of the fish. A symbolic reward consisting of a t-shirt or a cap was delivered or sent by mail for each tag returned. To improve the quantity and quality of the information received with the returned tags, a talk was given at an annual meeting of the local fishermen, and information on the work was provided

through local newspapers and fishermen's journals and letters to individuals who had returned tags.

Fish reported by fishermen were used for estimating dispersion while fish returned to the lab were used for estimating growth. From this information, we could deduce movements, number of days since release and growth. Specific growth rate (SGR % g d-1) was calculated using the initial/final weights following standard formulae (Steffens 1989). All catches using fishing rod and spear fishing were classified as recreational and all other gears were considered as commercial.

Results

Growth of hatchery produced and reared fish

D. cervinus larvae start feeding on *Brachionus* at day 3 after hatching (Figure 3) like *S. aurata*'s larvae but switch to *Artemia* at around 20DAH while *S. aurata* only switches nearly 5 days later. Weaning from live food to inert food occurs around day 21 for *D. cervinus* and only 4 to 5 days later for *S. aurata.*

The growth rates in the larval phase (up to 30 DAH) are higher than those of gilthead seabream (*Sparus aurata*) (Figure 4). *D. cervinus* and *S. aurata* final total length were 13.78±1.40mm and 7.04±0.04mm at 30DAH, respectively. The proportion of anomalies in the larva to juvenile stage is extremely low (11 out of 3200 observed fish, i.e. 0.3%). The anomaly typologies observed (after Boglione et al. 2003) were lordosis, saddle-back + kyphosis and lordosis + kyphosis.

Tagged fish results

For 200 out of the 8007 released fish, there was reported data on capture date and location, fishing gear used, size and/or weight, corresponding to an overall catch rate of

2.5%. The percentage of returns per batch ranged from 0.5% to 5.2% (Table 1). Local commercial and recreational fishermen contributed with all the captured fish. Fish were returned mostly by recreational fishermen (93%) out of which 11% were from spear fishers and 89% from rod-and-line anglers. The professional fisherman returned the remaining 7%, with the majority caught in fishing nets (62% from gill nets and 31% from trammel nets) and a single fish (corresponding to 8% of commercial catches) was captured in a fish trap.

The maximum observed days at liberty were 880 and the maximum travelled distance was 356 nmi. The average distance travelled per batch ranged from 8.1 to 43.4 nmi (see Table 1 for details). The analysis of the weight at capture (Figure 5) showed that there was an initial weight loss until 50 days after release (except for a single fish that was still under the initial weight after 69 days) and thereafter there was a continuous increase in fish weight. Nevertheless, 77% of the fish analyzed for the condition factor were in lower condition when captured than when released.

From the comparative results of the chronogram (Figure 6) and the captured fish characteristics (Table 2), it was noted that for the two released batches of medium sized fish (average greater than 100g) on consecutive days with fish of the same size at two different depth locations, returns were significantly higher for the batch released at the natural reef than those released near the breakwater. Releasing small fish (average weight less than 100g) near the artificial reefs at depth resulted in very few fish returns, while releasing a batch of larger fish on the same reef later in the year resulted in higher report rates. Finally, the highest return rate corresponded to a large batch of fish (nearly 3000), but the majority occurred during the 3 initial days after release (94 out of the total 156). The distance between capture location and release site did not show a direct increase over time with the average distance for each batch below 50 miles (Figure 7).

Discussion

Results of feeding schedules (Figure 3) for *D. cervinus* indicate an earlier ingestion of larger preys. This is particularly important from a production perspective as the costs for producing algae and *Brachionus* are particularly high since the producer is forced to maintain a stock of live food all year round, as opposed to *Artemia* which can be prepared from stocked dehydrated cysts. Weaning to inert food also occurs for *D. cervinus* at an earlier stage (3 to 4 days) which is also important from a cost/manpower perspective.

This species showed a better growth performance at the larval phase when compared to *S. aurata*. This is particularly interesting because the feed was optimized for *S. aurata*, which means that even better results could possibly be achieved with a custom diet. *D. cervinus* larval survival was in the expected range for Sparidae species (5-20 % from hatching to 30 DAH). Although *D. cervinus* showed slower growth during the on-growing phase (0.48% increase of body weight/day) compared with *S. aurata* (0.93% increase of body weight/day) (Pousão et al, unpublished), it has a higher commercial value and a small incidence or total absence of skeleton anomalies. This is particularly important from a production perspective when compared to *Diplodus sargus*, another Sparid species of even higher commercial importance in Portugal (see Lino et al, 2009). Even though the growth rate registered in pair-wise experiments between *D. sargus* and *D. cervinus* shows similar values, the percentage of malformed individuals of *D. sargus* at 180 DAH is between 14 and 36% (Dores et al, unpublished).

The results of the catches by recreational vs. commercial fisherman confirm the importance of this species for the recreational industry. This is related to the non-schooling behavior of this species, which makes it an occasional catch for commercial fishermen. Inversely, there is a targeted recreational fishery from chartered boats which

generates high catches for this species, since boats actively fish on rocky bottoms where this species occurs (Pajuelo, 2003a). According to a charter owner *D. cervinus* can account for up to 90% of the catch on such fishing trips (Soares, pers. com.)

The evolution of weight after release from the analysis of the reported captured fish indicates that as observed for *D. sargus* (Santos et al., 2006) there is an initial adaptation period during which the fish do not feed (due to behavioral deficit) which causes an initial weight loss. The fact that the condition factor of fish is lower when captured is most probably related to the fact that feeding frequency is much lower in the natural habitat than at the aquaculture station where feed is supplied *ad libitum*. In addition, even though the feed supplied is not optimized for the amino acid profile of *D. cervinus*, it is probably of much higher nutritional value than natural food items. Unfortunately it is not possible to compare the current data with wild specimens at a similar age due to the lack of published studies for this species. These results would probably show that the lower condition factor of the captured fish was not due to under feeding but to a convergence to the natural condition of wild fish.

The fish captures over time show that releasing *D. cervinus* at depth near a reef produces very little results in terms of captured fish, regardless of the size of fish (Batch 1 and Batch 2). However releasing large fish at the surface near a deep natural reef produced slightly better results and longer survival at sea (Batch 5). Releasing a larger batch (2 to 3 times larger) produces a non-proportional larger number of returns. However since most of the captures occurred in the 3 days following release, it shows that fisherman took advantage of the naiveté of the young reared fish, which contrasts with the much lower catches of larger fish released at the same location (Batch 4). The large number of fish caught could also reflect the higher boat-based recreational fishing effort that occurs during the summer months when compared to the effort occurring

during autumn. Nevertheless releasing batches of similar sized large fish near a breakwater was less effective than releasing them near a natural reef in terms of site fidelity in the long-run.

From a stock enhancement perspective the fact that most fish were captured within 50 nautical miles of the release location (i.e. along the South coast) and that this pattern was maintained up to nearly 500 days after release, showed that stock enhancement with hatchery reared fish can be effective at the local scale. These results show that *D. cervinus* has a higher dispersion than *D. sargus* or *S. aurata* (Santos et al, 2006) which were never reported beyond the South coast of the Algarve.

The single fish that was caught 880 days after release in Galicia (approximately 360 nautical miles distant in a straight line over the water) shows the resistance of the fish but could also indicate a deliberate migration. Tagged *Diplodus cervinus* were observed in pods mixed with wild (untagged) zebra seabreams near Portimão by scientific scuba divers (Bentes, pers comm.) which could provide some evidence that the hatchery released fish adapt to wild conspecific behavior. Thus, this movement to colder waters can be a species specific behavior which would be interesting to investigate from a management perspective.

The results from this study seem to indicate that the best option for effective stock enhancement action with *Diplodus cervinus* is to release small fish (around 15cm total length which corresponds to an age of one year) at the surface near a natural reef, during autumn in order to avoid the higher recreational boat-based fishing effort during summer. Sánchez-Lamadrid (2002) carried out a similar study with *S. aurata* in the Bay of Cadiz and reached the same conclusion, but recommended that fish should be released at the end of the summer in order to avoid the fishing pressure that occurs

during the summer but as early as possible to take advantage of the higher water temperature which stimulates feeding and growth.

From a cost/benefit perspective it would be important to release fish at a smaller size (i.e. less days in production and therefore lower cost of production). However, this hypothesis could not be tested for survival effectiveness since previous studies with tagged wild fish under the minimum legal size carried out on several species of Sparidae by Erzini et al.(2002) and with hatchery reared *Diplodus vulgaris* and *D.sargus* by IPIMAR (unpublished data) produced nearly no fish returns. Sánchez-Lamadrid (2002) reported success in fish returns with *S. aurata* of 100g but not with smaller fish of around 15g. The author related the lack of success with predation by birds, as reported by Olla et al (1998) and with the difficulty of fishermen in detecting the small tags used (Sánchez-Lamadrid, 2004)

This study provides some evidence that as a management tool, stock enhancement of this species can provide positive results. Obviously this can only work within an integrated management plan for local fisheries enhancement. If fishing activity moves from a sea harvesting perspective to a sea farmer perspective, fishermen organizations could have a role in the organized, scientifically assisted restocking of native species.

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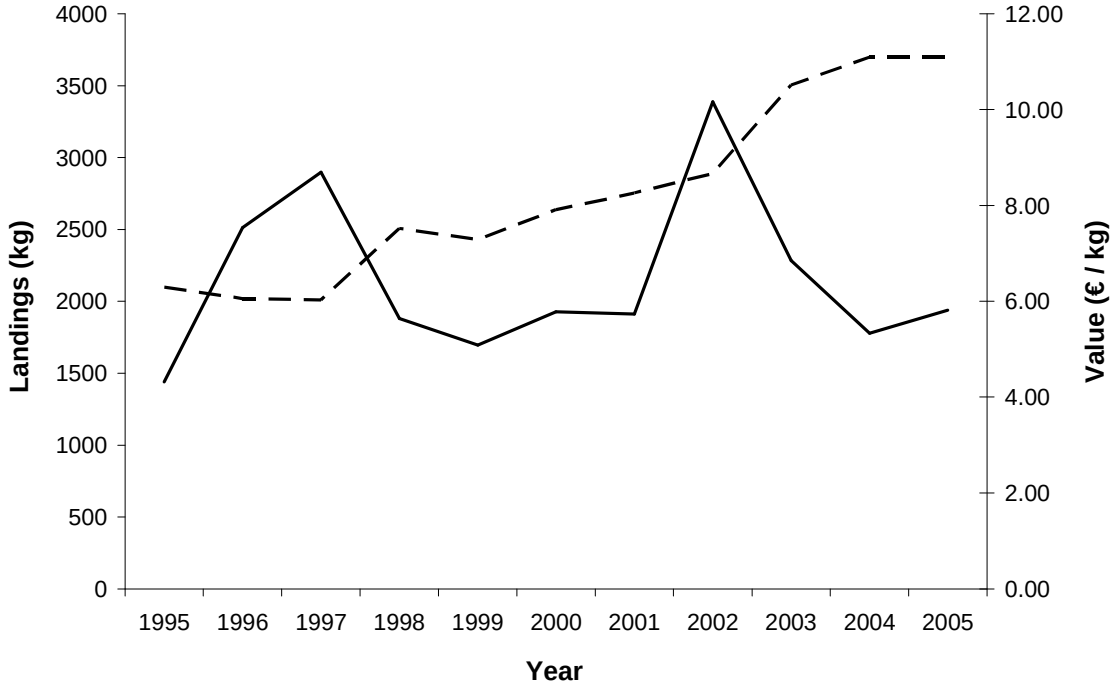

Figure 1 – Trend of landings in weight (full line, scale on the left) and of commercial value (dashed line, scale on the right) of *Diplodus cervinus* at first sale (fish auction) between 1995 and 2005.

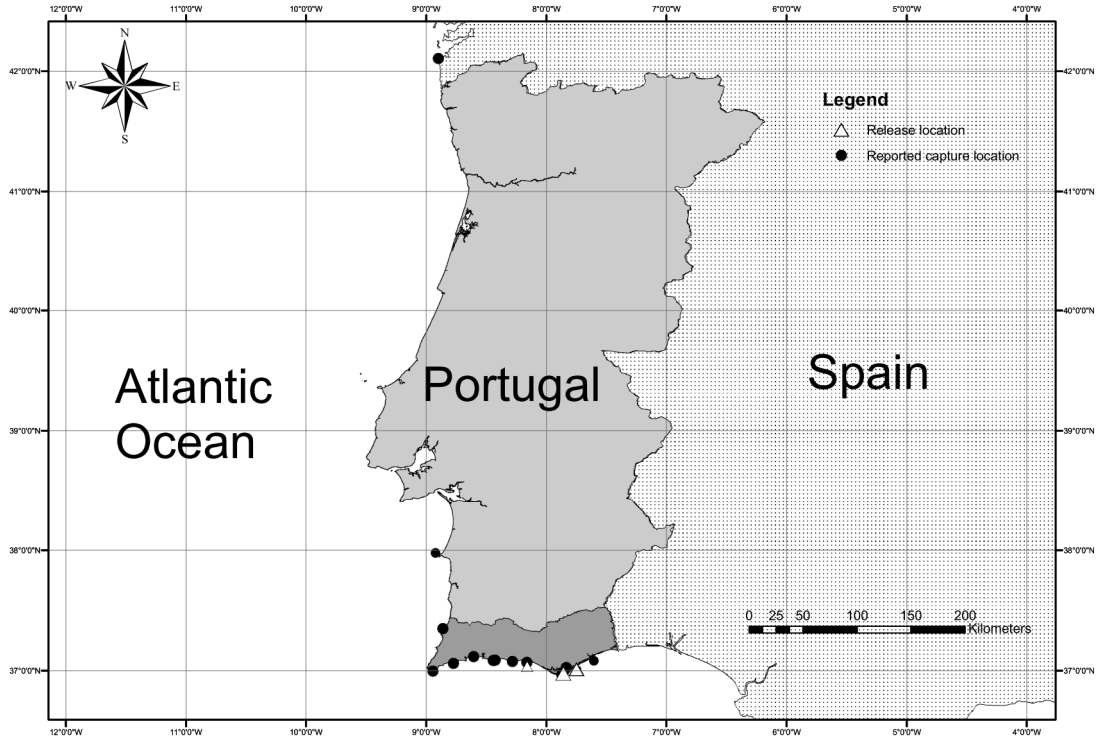

Figure 2 – Map of release and capture locations for the hatchery produced and reared zebra sea breams (*Diplodus cervinus*). The open triangles represent the release locations and the closed circles the capture locations.

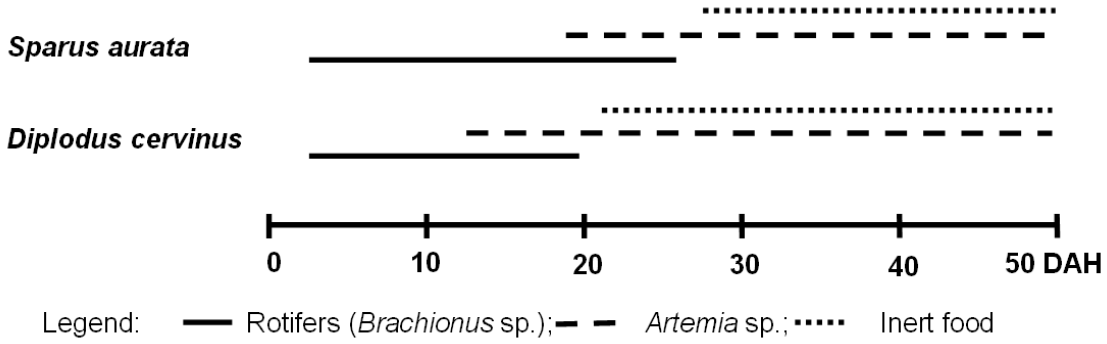

Figure 3 – Comparative feeding schedule of *Sparus aurata* and *Diplodus cervinus*. The bottom axis represents time since hatching (DAH = Days After Hatching). The full line represents the period when rotifers (*Brachionus* sp.) are supplied, the dashed line when *Artemia* sp. is supplied and the dotted line indicates the beginning of the inert food diet.

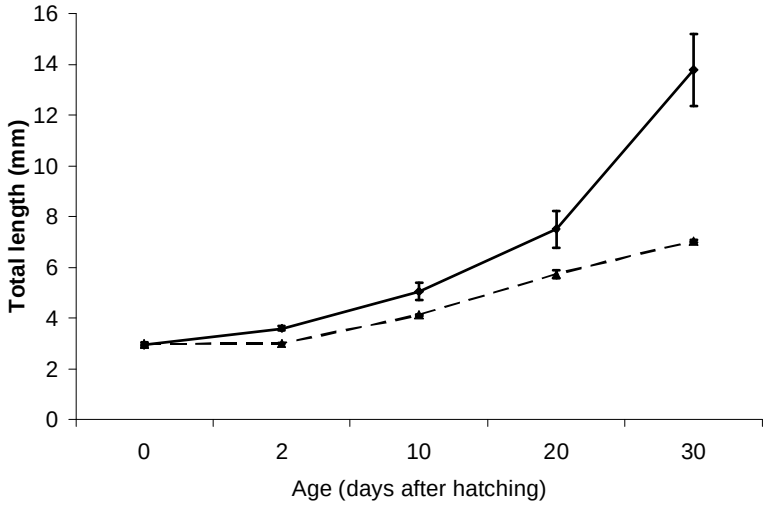

Figure 4- Growth in length of larvae of *Diplodus cervinus* (full line) and of *Sparus aurata* (dashed line).

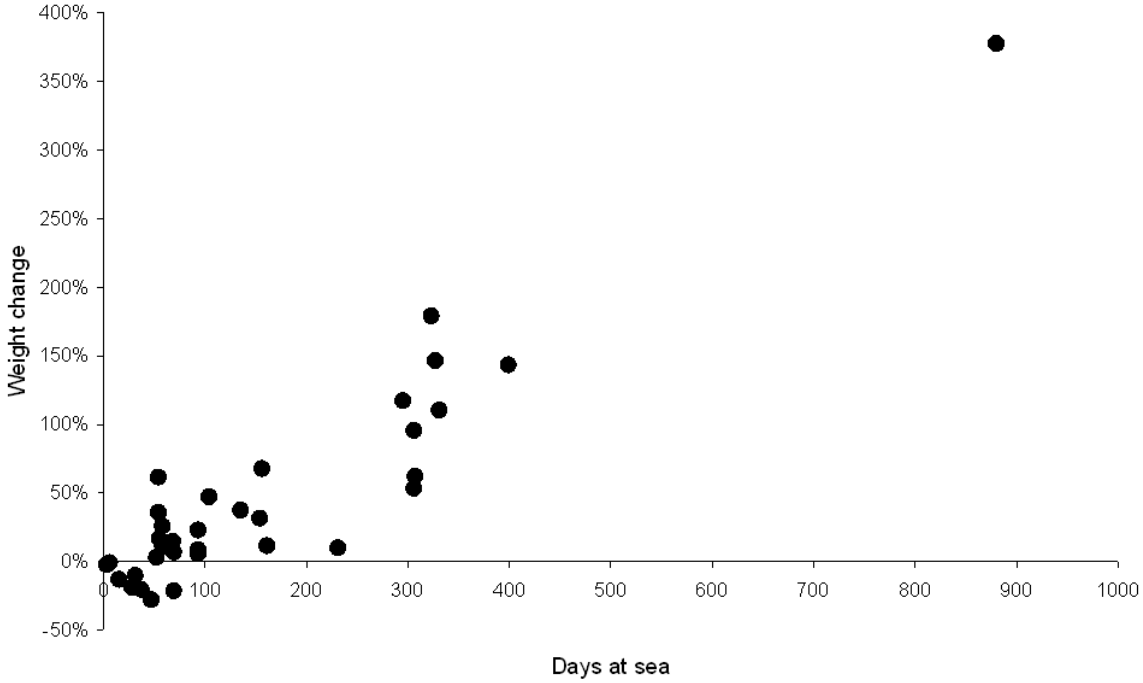

Figure 5 –Weight change of captured *Diplodus cervinus* as percentage of initial weight.

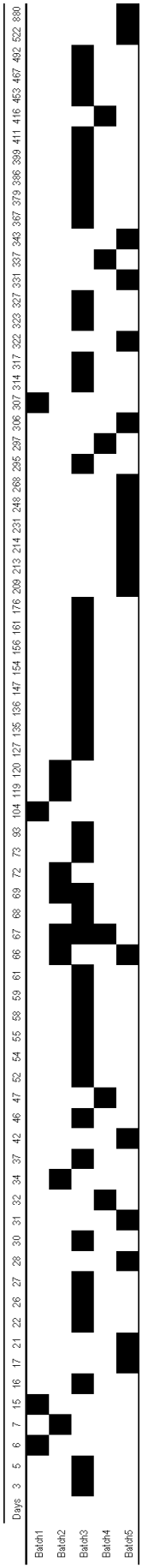

Figure 6 – Chronogram of captured *Diplodus cervinus* per batch

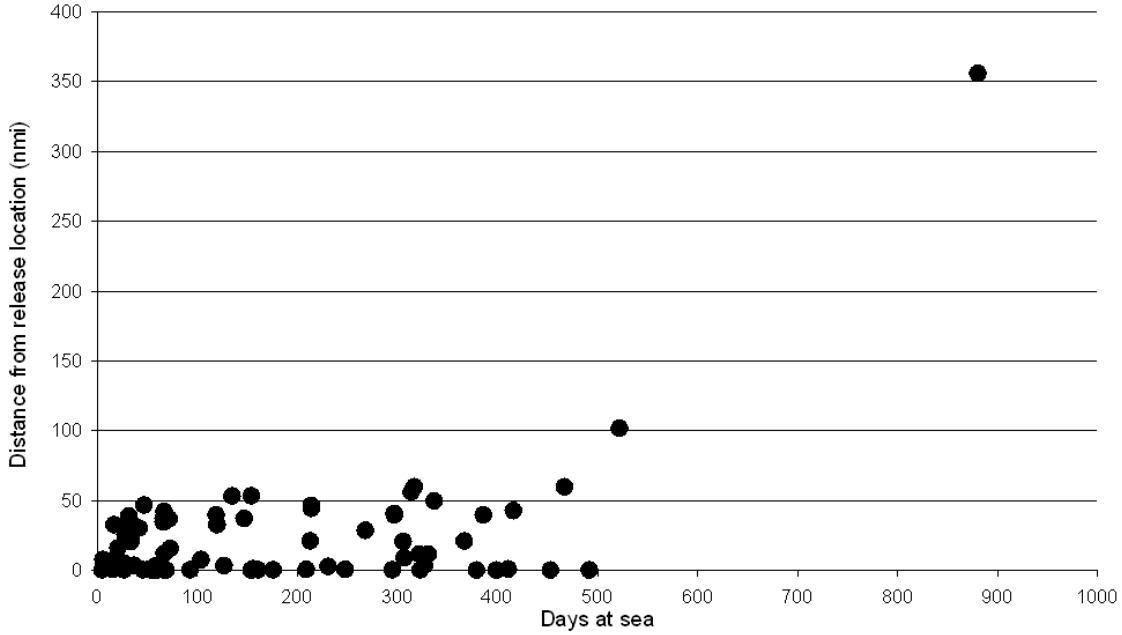

Figure 7 – Distance of the reported capture location (in nautical miles) to release site plotted against time since release (in days).

Table 1 – Characteristics of the batches of hatchery produced and reared *Diplodus cervinus* released at sea

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Release date	09-07-2004	16-11-2004	08-08-2005	16-11-2005	17-11-2005
# of fish released	1091	1110	2981	1409	1416
Total # of fish released			8007		
Total weight of batch (kg)	73.6	143.8	263.1	227.6	230.3
Total weight of released fish (kg)			938.3		
Release location	Olhão AR	Olhão AR	Near breakwater	Near breakwater	Natural reef
Depth	20m	20m	3m	3m	20m
Min of Furcal Length (cm)	11.2	13.1	12.0	12.1	13.0
Average of FL (cm)	13.3	16.5	14.8	17.8	17.9
Max of FL (cm)	15.6	19.8	17.6	20.7	21.3
Min of Total Weight (g)	38.0	68.0	44.0	53.0	54.0
Average of TW (g)	67.4	129.5	88.9	161.9	162.7
Max of TW (g)	115.0	233.0	144.0	260.0	265.0

Table 2 – Characteristics of the captured *Diplodus cervinus* released at sea

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
# of fish released	1091	1110	2981	1409	1416
Total # of fish released			8007		
# of fish captured	5	11	156	7	21
Total # of fish returned			200		
% returns	0.5%	1.0%	5.2%	0.5%	1.5%
Total % returns			2.5%		
# of fish with biological data	4	1	24	1	7
Max Days at sea	307	120	492	416	880
Max Distance travelled (nmi)	9.3	40	60	50	356
Average Distance travelled (nmi)	8.1	31.5	3.6	43.2	43.4
% Fish captured at < 10 nmi	80%	0%	88%	0%	14%

CHAPTER 6

Comparative behavior of wild and hatchery reared white sea bream (*Diplodus sargus*) released on artificial reefs off the Algarve (southern Portugal).

Authors: Pedro G. Lino, Luís Bentes, David Abecasis, Miguel Neves dos Santos and Karim Erzini

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Abstract

Three hatchery produced and reared (HPR) and five wild white sea bream (*Diplodus sargus*) were double tagged with Vemco V8SC-2L acoustic transmitters and Floy T-bar tags, and released on artificial reefs located near a natural reef off the southern coast of Portugal. Passive telemetry was used to monitor movements of the white sea bream over a nine week period from April to June 2007. Differences in behavior at release, habitat association (artificial vs. natural reef), and in daily movements were registered. Wild fish moved from one habitat to the other with increased preference for the artificial habitat during the day, whereas HPR fish showed no site fidelity or consistent daily movement pattern and left the release site soon after release. Comparison of Minimum Convex Polygon (MCP) showed a higher area usage by wild fish. This experiment shows that these artificial reefs are used on a daily basis by wild white sea bream but apparently are not optimal release locations for hatchery produced white sea bream.

Introduction

The white sea bream (*Diplodus sargus* Linnaeus, 1758), is a common species in the Eastern Atlantic Ocean and Mediterranean Sea (Whitehead et al. 1984). It is a highly valued species in Portugal, where catches have been declining since the late 1980s. Since 2001, IPIMAR has been carrying out restocking trials with fish produced and reared at the IPIMAR Aquaculture facilities (EPPO) in Olhão. Previous studies based on conventional tagging (T-bar anchor tags) and underwater surveys showed that reared specimens do not remain near the artificial reefs for long periods (Santos et al. 2006). However, these findings are limited by the reduced spatial coverage of underwater surveys and the data from conventional tagging, which provides no information on the behavior of the released fish between release and recapture events. Although underwater observations (Santos et al. 2006) showed that restocked white sea bream tend to school with similar sized wild specimens, it is not known if they have the same patterns of habitat use.

Acoustic telemetry is an ideal tool to address questions of movement and activity patterns of fishes (Zeller, 1999), with the latest transmitters being small enough to be implanted in fish weighing as little as 70g (Vemco, 2008) while respecting the 2% Tag : Body Weight Ratio (TBWR) rule of thumb. Although acoustic telemetry has been widely used in the marine environment to track fish movements and resolve habitat use, it has rarely been applied to compare habitat use of stocked hatchery-reared and wild fish (Taylor et al. 2006).

Age and growth, feeding ecology and reproduction of this commercially valuable species have been extensively studied (Man-Wai and Quignard 1982, Rosecchi 1987, Pajuelo and Lorenzo 2002, Lloret and Planes 2003). Other studies on this species

indicate that wild *Diplodus sargus* are resident species (Santos et al. 2005) on artificial reefs (AR), displaying site fidelity and using AR as a refuge (Pepe et al. 1998) and as feeding locations (Leitão et al. 2007). However, little is known about white sea bream daily movements and how this species uses its habitat.

Behavior of cultured fishes following release has important implications for their survival, growth, and reproduction and therefore for the outcome of restocking programs (Huntingford, 2004). The use of acoustic telemetry allows for data collection that can lead to a better understanding of the species ecology, namely the home range, habitat association and daily movements, which can be useful for improving conservation and management (Parsons et al. 2003) of the wild stocks and for optimization of restocking actions.

There are few published examples of the use of acoustic telemetry to investigate the movement patterns of Sparidae (e.g. Jadot et al. 2002, Parsons et al. 2003, Egli and Babcock 2004, Jadot et al. 2006). To the best of our knowledge there are no studies from Portugal, where several species of this family are particularly commercially important and where a restocking pilot project of native Sparidae species has been under way since 2001.

The main objective of this study was to compare the movement patterns of hatchery reared *Diplodus sargus* with those of wild caught specimens when released at 20m depth on an artificial reef. In addition to some aspects related with surgery methodology and handling optimization, the main foci were on: i) behavior of fish during and after release; ii) habitat association; iii) daily movements; and iv) area usage.

Material and methods

Fish used in this study were from two sources: hatchery produced and reared juveniles of *Diplodus sargus* from IPIMAR's Fish Production Unit and wild fish of the same species captured by longline within the study area. The study area is located in the southern coast of Portugal, at depths between 15 and 30 meters (Figure 1). This area is composed of two different sets of hard structures: a natural reef, extending for 3 km and the Faro artificial reef, consisting of several groups of concrete blocks placed at greater depths, seaward from the natural reef, and extending for 8 km.

Wild *Diplodus sargus* were caught with a baited longline with 100 hooks. The longline was constructed and operated in accordance with local gear specifications (Erzini et al. 1996) by a local fisherman contracted for the study. Hooks were baited with razor shell clam (*Ensis siliqua*) and the gear set near the seaward edge of the natural reef at day break and hauled regularly every hour until there were few baited hooks left. Fish were slowly hauled to the surface, unhooked and immediately anesthetized. Fish with an inflated bladder were punctured with a hollow needle and carefully massaged until they could swim upright.

HPR fish were the offspring (F1) of a wild caught broodstock. The fish were selected to comply with the 2% TBWR rule recommended by several authors (Jadot et al. 2005), since no previous studies were made for this species.

All fish were double tagged with a Vemco V8SC-2L acoustic transmitter, surgically implanted in the abdominal cavity, and a Floytag T-bar anchor tag below the dorsal fin. Both wild and HPR fish were anesthetized in a 0.4 ml/l 2-phenoxy-ethanol solution. When the fish were fully anesthetized, showing no reaction to external stimuli (1-2 min), they were measured (Fork Length and Total Length in cm). HPR fish were also weighed to the nearest gram. The TBWR for the HPR fish ranged from 1.4 to 1.7%. The weight for the wild fish was estimated using the weight-length relationship published by

Gonçalves et al. (1997) and the TBWR ranged between 0.7 and 1.5%.

Fish were placed in a V-shaped berth, with a 0.2 mg/l 2-phenoxy-ethanol solution being pumped into the fish's mouth. An incision (~1.5cm long) was made on the mid ventral-line, posterior to the pelvic girdle, and the transmitter (disinfected in povidone iodine) was inserted in the peritoneal cavity. On a control HPR batch the wound was closed with one or two individual sutures using nylon monofilament (Braun Dafilon 3/0 DS19 45 cm) and cutting needles. Cyanoacrilate adhesive (Vetseal, B. Braun Medical, Sempach) was used to close the incision and to consolidate the knots. On all other batches the incision was closed with cyanoacrilate adhesive only. The duration of the surgery was under 2 minutes for each fish.

Hatchery reared fish were placed in a clean holding tank at the IPIMAR aquaculture facilities and monitored for infection and/or tag loss. Wild fish were placed in a holding tank alongside the boat with clean sea water flowing through, until they regained equilibrium (less than 2 minutes).

Fish were released at 20m depths on the Faro artificial reef by lowering them in two transport cages (one for wild fish and another for HPR fish), held by scuba divers who constantly monitored their condition during descent. The cages were opened simultaneously at different points on the reef.

The experimental design aimed to maximize the acoustic coverage of the sampling area. An array of 13 VR2 (Vemco) hydrophones was used to track the movements of the tagged fish over an extensive area (10.2 km^2) of both natural and artificial reefs. Two rows of receivers were set, with the first located between the natural reef and the artificial reef, and the second among the artificial reef groups. Concrete filled tires and concrete blocks were used to anchor the VR2 receivers and the locations were recorded by GPS. Passive acoustic sampling extended over a period of 9 weeks, from April to

June 2007.

The Minimum Convex Polygon (MCP) area was estimated using the MCP function included in ArcGis extension Hawth's Analysis Tools v3.27.

Results

Fishing with the longline gear took place on the April 19, with five white bream tagged and released on the same day. Three HPR previously tagged were released simultaneously as the wild fish (Table 1). Wild white sea bream were larger than the HPR fish, ranging from 28.9 to 34.2 cm in total length (TL), while HPR fish were 25.7 to 27.0 cm TL (Table 1).

Surgery and fish behavior during transportation and immediately after release

The experiment was quite successful in optimizing handling and surgery time. One batch of 3 HPR fish had their incisions closed with one individual suture and cyanoacrilate, as suggested by the literature (Jadot et al. 2005), while cyanoacrilate alone was used on the second batch. This first group of fish was held under observation for 50 days and was never released. The second group was held for 3 days during which there were no signs of infection and no tag loss. The use of cyanoacrilate alone was also used with the wild fish to simplify procedures on-board the fishing boat.

The fish showed contrasting behavior during transport to the release depth, with hatchery reared fish always swimming towards the surface, while wild fish swam down towards the bottom. When the transport cages were opened, the wild fish immediately swam out, seeking refuge in the artificial reefs while hatchery reared fish refused to

leave the cage. When they were forced to exit the cage, some of the HPR fish tried to return inside.

Habitat association

The chronogram shows that the wild fish have a clear pattern of use of the natural reef with almost every fish being present in the area during the study period (Figure 2). For the artificial reef, the habitat use was intermittent, particularly in the last quarter of the study period, showing that for each individual there was an association with the natural reef, with the exception of individuals #126 and #128 which visited both habitats daily.

The HPR fish showed no consistent pattern of habitat association. One specimen (#163) remained in the artificial habitat and then left the study area, while another specimen (#162) did the opposite and a third (#164) left the study area immediately after release, heading towards the coastline in a northerly direction, instead of taking the closest path in a North-East direction.

Daily movements and area usage

There was a clear daily movement pattern for the wild fish within the studied area, particularly noticeable on the artificial reefs. The daily movement cycle started about one hour before sunrise and ended by or a few minutes before sunset (Figure 3a). Despite a regular circadian rhythm for wild fish, HPR fish did not show any consistent daily patterns (Figure 3b). The reduction of nocturnal detections for both groups of fish could be explained by a migration to areas out of the range of the acoustic receivers or by the fish sheltering in caves at night, thereby limiting detection.

The MCP area (mean±SD) was 0.63±0.09 km^2 for the HPR fish and 1.61±0.89 $km^2$for the wild fish (Table 1). The mean MCP areas for the two groups were not significantly different (Mann-Whitney Rank Sum Test, U=8.500, p=0.190).

Discussion

In terms of surgery methodology, this experiment was quite successful in optimizing handling and surgery time. The use of cianoacrylate alone reduces handling time and appears to have no negative effects. The long time track of the wild fish movement proves that the surgery was successful and not lethal to the fish (at least for the duration of the study).

Hatchery fish released under the current conditions showed no clear movement pattern. Two different results were observed: a) leaving almost immediately towards the coastline, b) remaining in the area 2-4 weeks and leaving thereafter. The observed behavior of the hatchery reared fish is consistent with the underwater observations reported by Santos et al. (2006).

The behavior of the hatchery reared fish is not unexpected since they were reared in shallow tanks, exposed to intense daylight and expected their food to come from the surface. Uglem et al. (2008) also found the same differences between wild caught and hatchery reared cod (*Gadus morhua*) deliberately released to simulate a cage escape. As in this study, hatchery reared fish dispersed rapidly, in no particular direction. Wild cod remained in the same general area where they were caught, much like the sea bream in our study.

In a previous telemetry experiment carried out by this team (unpublished data) with 4 tagged HPR *Diplodus sargus* released on another artificial reef, the longest site fidelity

in the release area was 31.5 hours. The other 3 fish remained 45 minutes, 1 hour and 2.5 hours before moving in different paths towards the coast or shallower waters. However, unlike the present study, the artificial reefs were located on a sandy bottom area with no natural reefs in the vicinity. The results of these two experiments seem to indicate that the presence of a natural, more complex habitat in the vicinity of the release location might increase site fidelity in the short term, even if it is a suboptimal habitat.

Hatchery-reared fish show deficits in virtually all aspects of behavior due to the impoverished conditions in which they are raised (Brown and Laland 2001). According to the same authors, hatchery fish that are many generations removed from their wild counterparts are likely to have more impoverished life-history skills and may take longer to train than those separated by fewer generations. However, this was not the case with the HPR fish used in this study since they were all F1 (first generation) from a wild broodstock. On the other hand, the differences in behavior seem to increase with the proportion of life spent in captivity (Svasand et al. 2000). This is an expected effect but since it is not possible to tag smaller fish due to battery size/duration limitations, there is currently no technical solution for this dilemma.

From an energetic point of view, it would be interesting to determine if wild white sea bream reduce their movements during the night or if they perform daily migrations to other grounds. Diel behaviors and movements of fish have been reported in many fish species (Yokota et al. 2007), and particularly for some *Diplodus* species (Santos et al. 2002). However, these daily variations in movements were less obvious for HPR fish. This would not be surprising if the lack of detections at night is due to reduced activity and use of caves, since HPR fish would not be adapted as they are forced to swim continuously in the aquaculture tanks and have no crevices or caves to rest in. Further experiments with this species are scheduled to test the migration versus inactivity

hypothesis.

The wild fish used the whole study area with preference for the natural reef. It is interesting from a management point of view to note that they perform daily migrations to the artificial reef. HPR fish did not show a preferential association with any of the habitats.

The MCP values were not statistically different between the two groups of fish. However, they show a wider use of the study area by the wild fish. This is to be expected since they were released in familiar territory, compared to the HPR fish, which were released in a totally unfamiliar environment. The MCP values for the wild fish were greater than those reported for other similar sized sparidae such as *Sparus aurata* (Abecasis and Erzini, 2008). However, the latter study was for a lagoon habitat, characterized by extensive channels. Since the tagged fish eventually left the lagoon and were not detected further, the mean MCP of 0.17 km^2 should only be considered valid for the juvenile part of the life cycle.

The short residence time and reduced area usage of HPR fish released on these artificial reefs seem to indicate that this is a suboptimal habitat and that releasing fish for restocking purposes on this location may not appropriate. It is therefore important to assess whether and to what extent present knowledge of the developmental origin of behavioral deficits in cultured fishes can be combined with programs of habitat improvement to make restocking programs more effective (Huntingford, 2004). Further studies on the adaptation of HPR *Diplodus sargus* are needed to improve their survival in the wild. These include improved migratory, anti-predator and feeding behavior in hatchery fish, as suggested by Brown and Laland (2001) and based on our findings, also by improved daily activity adaptation. Acclimation to the release location using holding cages or pre-adaptation to an artificial habitat that is moved to release site as well as

increasing artificial reef complexity are strategies to be considered in further experiments.

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Table 1. Characteristics of wild and hatchery produced and reared (HPR) white sea bream, surgery and release dates, and minimum convex polygon. ID is the identification number returned by the pinger, TL is Total Length, TW is Total Weight, and MCP is the Minimum Convex Polygon. NA means the value could not be calculated.

ID	Source	TL (cm)	TW(g)	Surgery	MCP (km^2)
113	Wild	29.6	464	19-04-2007	0.697
124	Wild	34.2	733	19-04-2007	2.557
126	Wild	28.9	430	19-04-2007	0.609
127	Wild	31.7	577	19-04-2007	2.104
128	Wild	31.1	543	19-04-2007	2.074
162	HPR	25.7	313	16-04-2007	0.697
163	HPR	26.8	294	16-04-2007	0.571
164	HPR	27.0	303	16-04-2007	NA

Figure 1. Location of natural reef, artificial reefs, and VR2 hydrophones off the southern coast of Portugal. The black square in the inlay picture shows the location of the study area.

Figure 2. Detection patterns of the tagged hatchery produced and reared and the wild fish on the natural and artificial reefs. Shaded areas indicate presence.

Figure 3. Daily patterns of habitat use on artificial and natural reefs: a) wild white sea bream, b) hatchery produced and reared white sea bream. The dotted area corresponds to sunrise/sunset and the dashed area corresponds to the night period.

Figure 4. Minimum Complex Polygon (MCP) of the wild (a – e) and the hatchery produced and reared (f - g) white sea bream.

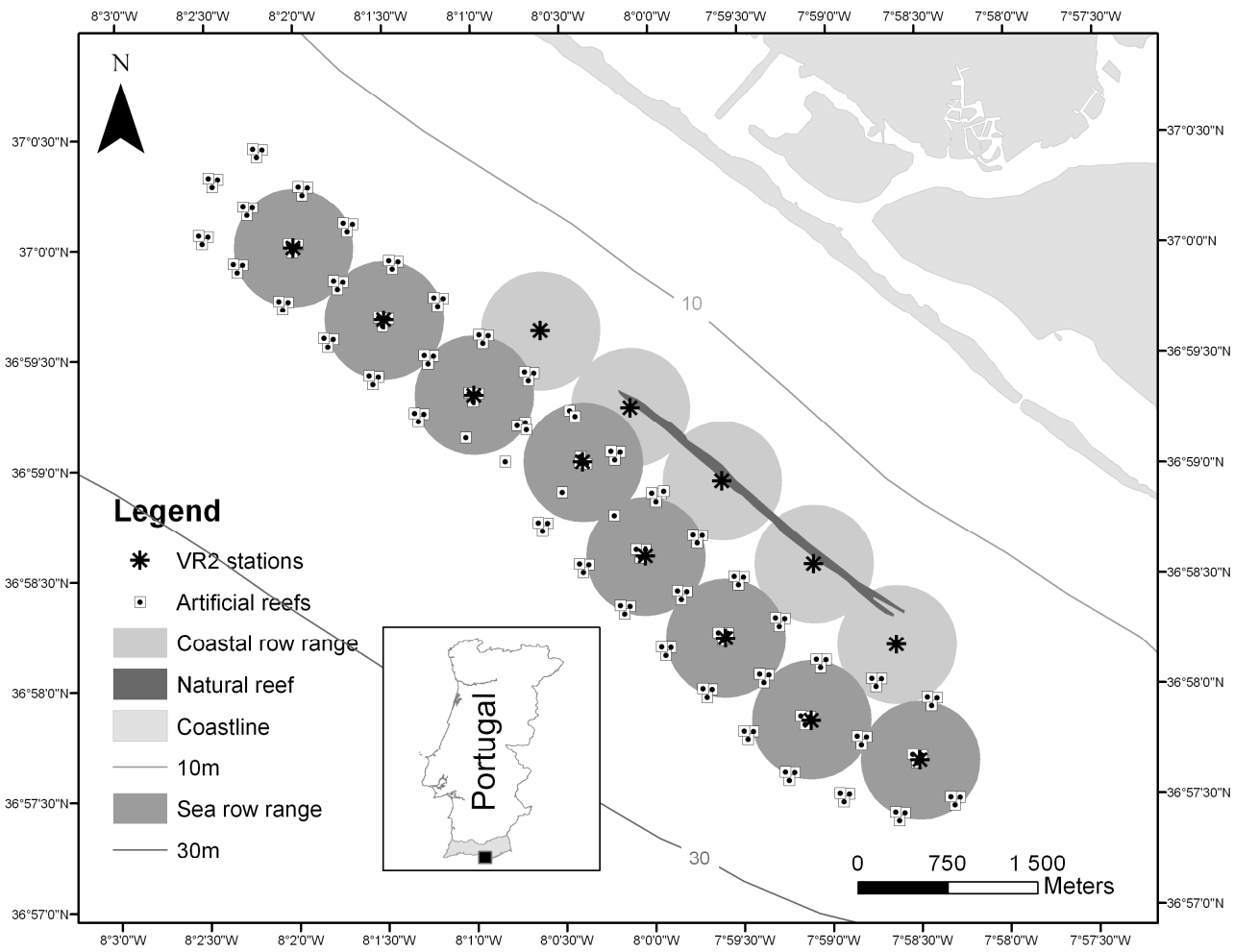
8°3'0"W
8°2'30"W
8°2'0"W
8°1'30"W
8°1'0"W
8°0'30"W
8°0'0"W
7°59'30"W
7°59'0"W
7°58'30"W
7°58'0"W
7°57'30"W
N
37°0'30"N
37°0'0"N
36°59'30"N
36°59'0"N
36°58'30"N
36°58'0"N
36°57'30"N
36°57'0"N
10
30
Legend
VR2 stations
Artificial reefs
Coastal row range
Natural reef
Coastline
10m
Sea row range
30m
Portugal
0
750
1 500
Meters

Figure 1

FISH ID
Reef
April
May
June
19 20 21 22 23 24 25 26 27 28 29 30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
113
Artificial
Natural
124
Artificial
Natural
126
Artificial
Natural
127
Artificial
Natural
128
Artificial
Natural
162
Artificial
Natural
163
Artificial
Natural
164
Artificial
Natural

Figure 2

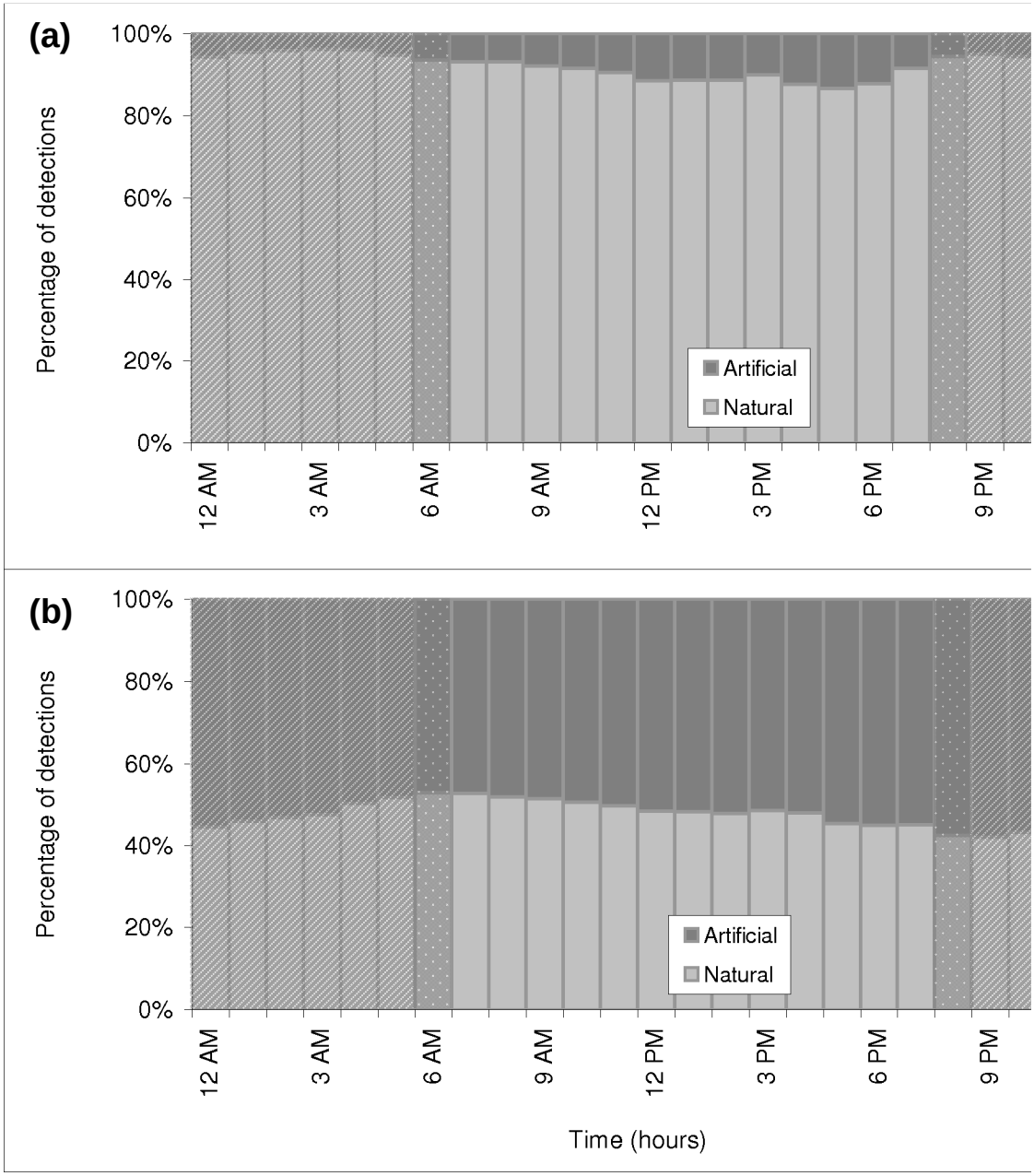
(a)
100%
80%
60%
40%
20%
0%
Percentage of detections
Artificial
Natural
12 AM
3 AM
6 AM
9 AM
12 PM
3 PM
6 PM
9 PM
(b)
100%
80%
60%
40%
20%
0%
Percentage of detections
Artificial
Natural
12 AM
3 AM
6 AM
9 AM
12 PM
3 PM
6 PM
9 PM
Time (hours)

Figure 3

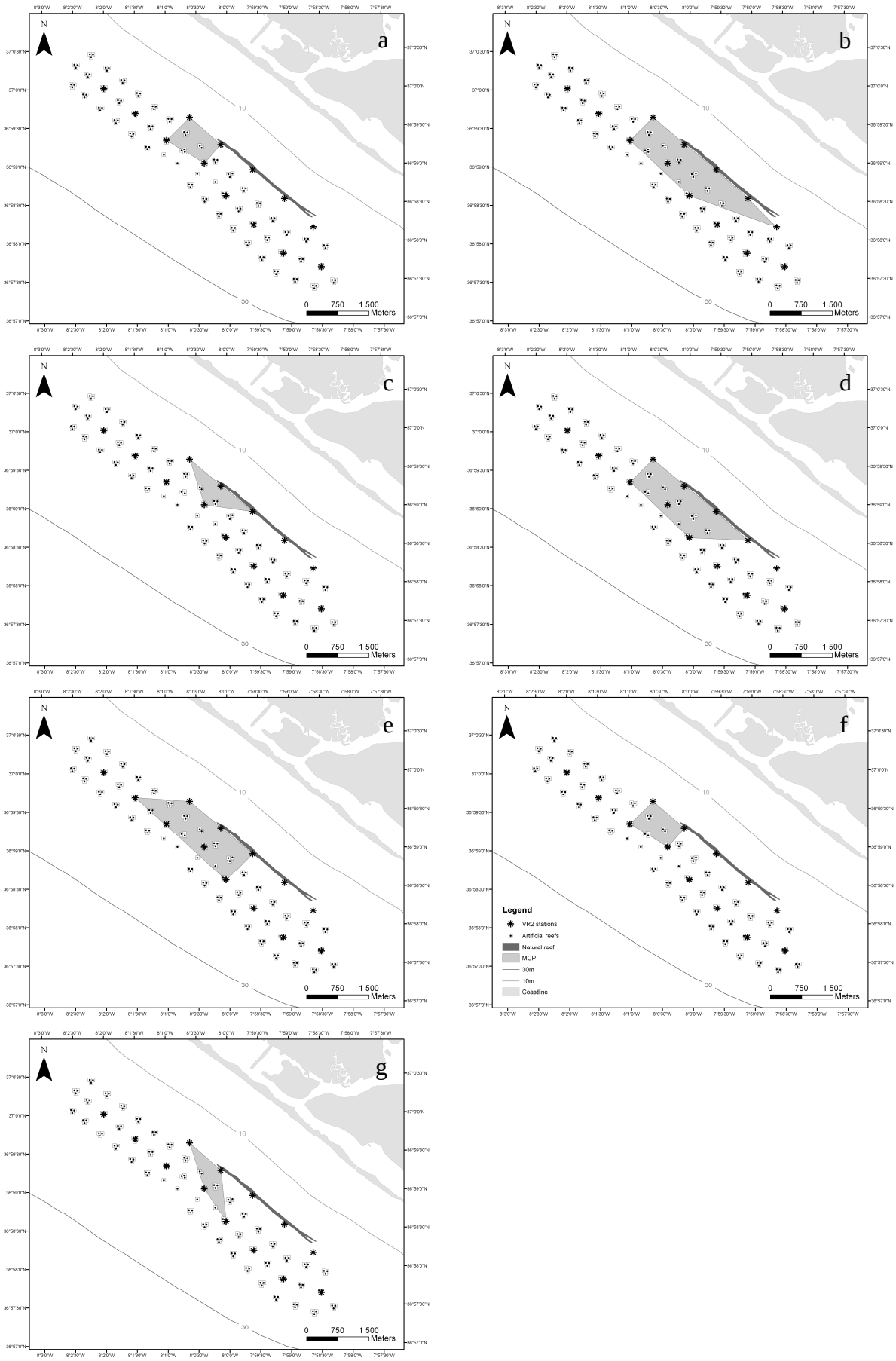
a
b
c
d
e
f
g
N
10
30
0 750 1 500
Meters
Legend
VR2 stations
Artificial reefs
Natural reef
MCP
30m
10m
Coastline

Figure 4

CHAPTER 7

Effect of cage acclimation on the dispersion of two species of hatchery produced and reared sea breams (*Diplodus sargus* and *D. cervinus*) off the South coast of Portugal.

Authors: Pedro G. Lino, Luís Bentes, David Abecasis, Miguel Neves dos Santos and Karim Erzini

Status: To be submitted

Abstract

Restocking trials with hatchery produced and reared sea breams have been carried out by IPIMAR since 2001. One of the factors affecting restocking is adaptation to the release location. White seabreams (*Diplodus sargus*, Sparidae, Perciformes) and zebra seabreams (*Diplodus cervinus*) hatched and reared at the IPIMAR's Fish Production Unit were tagged with VEMCO brand V8SC "coded" pingers. An array of 15 VEMCO brand VR2/VR2W acoustic receivers was set off the south coast of the Algarve (southern Portugal). The comparison of the movements of 10 hatchery reared fish, 5 of each *Diplodus* species, when released at 20m depth, near an artificial reef, 2 half acclimated for 2 days and 3 released immediately showed that cage acclimation had a negative effect on site fidelity. Non-acclimated fish showed a daily pattern of activity with high activity between sunrise and sunset. Acclimated *D. sargus* preferred the shallow area while non-acclimated fish of both species preferred the natural reef area. Acclimated *D.cervinus* left the study area briefly after release. The indexes proposed to evaluate use of the area (I_{rw}) and of relative movement (DTI) seem to provide extra information on the activity of the fish within the study area.

Introduction

Since 2001, the Portuguese Fisheries and Marine Research Laboratory (IPIMAR) has been carrying out restocking trials with fish produced and reared at the IPIMAR Aquaculture Research Station (EPPO) in Olhão. Previous results based on conventional tagging (T-bar anchor tags) and underwater surveys showed that reared specimens do not remain near the artificial reefs for long periods (Santos et al. 2006). A

previous study (Lino et al. 2009) carried out in the same area using a smaller array of receivers provided some answers but raised the question if acclimation to release site would improve site fidelity.

The successful production of gilthead seabream (*Sparus aurata* Linnaeus, 1758) in aquaculture has created the methodology to produce other Sparidae, offering a wider variety of species for human consumption (Alarcón and Carmen-Alvarez, 1999), but also opportunities for releasing cultured juveniles into the wild with the aim of increasing fishery recruited populations and catches (Bell et al., 2006). According to Støttrup and Sparrevohn (2007) the potential for stocking of a given species is derived from several factors, including the capacity to produce fish in sufficient quantities. In recent years, the EPPO has managed to achieve mass production and rearing of several Sparidae species, namely *Diplodus sargus*, *D. cervinus*, *D. vulgaris* and *D. puntazzo*.

The white seabream (*Diplodus sargus* Linnaeus, 1758) and the zebra seabream (*Diplodus cervinus* Lowe, 1838) are two common species in the Eastern Atlantic Ocean and Mediterranean Sea (Whitehead et al. 1984). Both are highly valued species in Portugal, where catches have been declining since the late 1980s. The white sea bream is a schooling species with opportunistic feeding behavior (Figueiredo et al, 2005) while the zebra sea bream lives in small pods and has a selective preference for amphipods and polychaetes (Lechanteur and Griffiths, 2003)

Although there are a few studies on the biology of *D. cervinus* from the Canary Islands (Pajuelo et al, 2003a and 2003b; Dominguez-Seoane, 2005) and from South Africa (Lechanteur and Griffiths, 2003; Mann and Buxton, 1992), there is no information on their *in situ* behaviour.

From a restocking point of view it is important that adaptation to the wild is done on a per species basis (Bell et al, 2006) It is therefore important to increase the

knowledge on the species behavior which can contribute to increase their survival, growth and reproduction (Huntingford, 2004). Furthermore, developing release strategies that minimize stress responses and increase post-release survival and site fidelity is essential to any stock enhancement program and can be done with a combination of hatchery and field techniques. One such technique is using acclimation cages *in situ* (Fairchild et al, 2010; Jonssonn et al, 1999). To the authors' knowledge this is the first study on the behavior of *D. cervinus* and the first study on acclimation of Sparidae for restocking purposes.

The main objective of this study was to compare the behavior of the hatchery produced and reared specimens of the two species of *Diplodus* when released at sea. The species specific responses and acclimation to release site influence were analyzed for: 1) habitat preference; 2) area usage; and 3) distance traveled.

Material and methods

The fish used in this study were hatchery produced and reared juveniles of *Diplodus sargus* and *Diplodus cervinus* from IPIMAR's Fish Production Unit. All fish used in this experiment were the offspring (F1) of a wild caught broodstock. The fish were selected to roughly comply with the 2% Tag to Body Weight Ratio (TBWR) rule recommended by several authors (Jadot et al. 2005).

The *D. sargus* specimens used (Table 1) were 23.4cm ± 0.31 SD in Total Length (TL) and 234.8g ± 16.50 SD in Total Weight (TW), while the *D. cervinus* specimens were 23.5cm ± 0.82 SD in TL and 256.0g ± 31.52 SD in TW (Table 1). There were no statistically significant differences between the four groups of fish, neither in length nor in weight (one-way ANOVA Length $F = 0.402$ $P = 0.756$; Weight $F = 1.517$ $P = 0.283$).

The TBWR ranged between 1.9 and 2.3% for *D. sargus* and between 1.7 to 2.2% for *D. cervinus*.

All fish were double tagged with a Vemco V8SC-2L acoustic transmitter, surgically implanted in the abdominal cavity, and a Floytag T-bar anchor tag below the dorsal fin on the left side. Fish were anesthetized in a 0.4 ml/l 2-phenoxy-ethanol solution. When the fish were fully anesthetized, showing no reaction to external stimuli (1-2 min), they were measured (Fork Length and Total Length to the nearest mm) and weighed to the nearest gram.

Fish were placed in a V-shaped berth, with a 0.2 mg/l 2-phenoxy-ethanol solution being pumped into the fish's mouth. An incision (~1.5cm long) was made on the mid ventral-line, posterior to the pelvic girdle, and the transmitter (previously cleaned in povidone iodine) was inserted in the peritoneal cavity. Cyanoacrilate adhesive (Vetseal, B. Braun Medical, Sempach) was used to close the incision. The duration of the surgery was under 2 minutes for each fish. Fish were placed in a clean holding tank at the IPIMAR aquaculture facilities and monitored for infection and/or tag loss. All surgeries were carried out in mid July 2008 allowing fish to recover for two weeks before the experiment started. No mortality or tag loss was registered during recovery.

A conditioning test was carried out at IPIMAR's Aquaculture Station where 3 *D. sargus* were placed in a fish pen submerged in an earthen pond with 2m depth. The fish pen (80x80x50cm) was constructed of an iron frame and plastic netting with a 3cm squared mesh. On one of the side panels a small (20x30cm) door allowed access to the fish. The fish used were the from the same size range to be used on the sea trials (around or above 250g to follow the 2% TBWR rule) so in this cage they were at a low fish density of under 2.5 Kg/m3. The fish were observed daily for injury and survival.

On day 5 one of the fish was observed to have an injured tail fin so the experiment was terminated. The experiment was repeated with 3 *Diplodus cervinus*. On the third day one of the fish showed damage on the tail fin so the experiment was terminated. Based on these results it was decided that two days would be the maximum time for leaving the fish in this type of cage.

For this experiment two fish pens were placed over the sandy bottom at 1m distance from the Faro artificial reefs at 20m depth. Fish were placed on the fish pens by lowering them in two transport cages (one for each species) held by SCUBA divers who constantly monitored their condition during descent. Each fish pen held 3 specimens of the same species for 2 days. At the end of the second day scuba divers transported down 2 cages containing 3 fish of each species and simultaneously released the four batches of fish at different points on the reef. The fish were released by simply opening the door of the fish pens and cages completely and allowing the fish to freely swim out.

The study area is located in the southern coast of Portugal, at depths between 15 and 20 meters (Figure 1). The bottom type in the area is mainly sandy and includes two different types of hard structures: a natural reef, extending for 3 km and the Faro artificial reef (AR), consisting of several groups of concrete blocks placed at greater depths, seaward from the natural reef, and extending for 8 km. An array of 15 VR2 (Vemco) hydrophones was set to track the movements of the tagged fish over an extensive area (14 km^2). Three parallel rows of receivers were set (Figure 1), with the first (Shallow) closer to the coastline at shallow depth (10-13m) consisting of 6 receivers, the second (Mid) located between the natural reef and the artificial reef, and the third (Deep) among the artificial reef groups. Concrete filled tires were used to anchor the VR2 receivers over the sandy and natural reefs and the locations were recorded by GPS. On the AR, the VR2 receivers were attached to a 1m long cable tied

to the upper reef modules and held vertically by a mid-water float. Therefore, except for Stations 1 to 4, all were set on soft sandy bottom. Passive acoustic sampling extended over a period of 10 months, from August 6th 2008 to May 25th 2009 with an effective monitoring period of 277 days.

The Minimum Convex Polygon (MCP) areas were estimated using the Animal Movements' Calculate MCP function included in ArcGis extension Hawth's Analysis Tools v3.27. A total MCP, minimum polygon area which includes all receivers was calculated to estimate the percentage used by each fish.

In this study the Residence Index (I_R) proposed by Afonso (2008) was included for comparison purposes but a weighted residence index (I_{WR}) was used. The I_{WR} accounts for the number of days the fish is detected (Dd) as a proportion of the total number of monitoring days (Dt) and is weighted by the interval in days between first and last detection (Di) as a proportion of the total number of monitoring days (Dt).

$$I_{WR} = \frac{D_d}{D_t} \times \frac{D_i}{D_t}$$

An estimated Distance Traveled Index (DTI) was calculated by adding the distances between the receivers the fish were sequentially detected by. If a fish was detected simultaneously by two receivers an intermediate position was calculated and the distance to that point added.

Results

Five white seabreams and five zebra seabreams (3 immediately released and 2 acclimated for 2 days) were released on the 6th of August 2008. One specimen of each

species died in the holding pen.

The passive telemetry lasted for 292 days but there were no further detections after January (Table 2). During this period a total of 237670 detections were received by the array of receivers . Only ST4 located on the Eastern edge of the Deep line of receivers did not register any detection (Table 3).

Comparative behavior

Cage acclimated fish remained in the study area less time than fish immediately released. In general *D. cervinus* specimens remained in the study area for less than one and a half months although specimen DC-NA1 returned at intervals.

Two of the non-acclimated *D. sargus* specimens remained within the study area for nearly 6 months while the third fish left the study area for long periods but returned for brief periods 4 and 5 months after release.

There was no statistically significant difference between the Weighted Residence Indexes of the 4 groups (One way ANOVA F=2.368; P=0.170) and there were no significant differences between the two species (t-test P=0.148; Power=0.292 for Alpha=0.05) or the two treatments (Mann-Whitney Rank Test U=4.000; P=0.114).

Habitat association

Non-acclimated *D. sargus* showed preference for the natural reefs, while acclimated *D. sargus* preferred the inshore, sandy bottom shallow area. The *D.cervinus* specimens did not stay long enough in the study area, but one non-acclimated fish showed preference for the Mid area where the natural reef was located.

Daily movements and area usage

D. cervinus showed a clear daily pattern of activity with high activity between sunrise and sunset. There seems to be a time lag between start and end of activity for the two treatment groups. Non-acclimated *D. sargus* showed a flat line pattern meaning they were equally active all day. The acclimated *D.sargus* showed no pattern.

There was no pattern of area usage but the majority of fish used a small proportion of the study area (Table 4). Fish Ds-NA1 which was detected by 10 receivers, moved one third of the DTI value observed for fish Dc-NA2 which was detected by the same number of receivers. Inversely fish Ds-A2 which was only detected by 7 receivers had the largest MCP (5.67 Km2) which corresponded to 79% of the total MCP. However the distance traveled was less than that corresponding to the fish detected by a larger number of receivers. Comparing the fish for which no MCP could be calculated (it is impossible to calculate an area with two points), it was possible to conclude that fish Dc-A1 was more active (moved twice the distance) than fish Dc-A2 and Dc-NA3.

Finally it should be noted that the last detection of 5 fish was at the NW limit of the study area (ST1 and ST9), 3 were last detected in a central area (ST8), 1 fish was last detected at the SE limit and another one last detected occurred in the shallow row (ST11) near to the coast.

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Discussion

Preliminary experiments conducted at the IPIMAR fish production station

showed that holding the fish for longer than two days was inappropriate. Simultaneously, control fish were held in a tank unfed to test for starvation effects. Since the caging experiment was terminated when visible injuries appeared, the starvation experiment was also terminated with no mortality. Therefore two days was considered the limit for caging duration.

Acclimation in the cages used in the present study proved to be inefficient since although no mortality occurred in the earthen ponds, one out of three specimens of each species died during acclimation *in situ*. This could have been caused by the stress of transportation to release site (Fairchild *et al*, 2010) in accumulation with caging and starvation since none of these factors acting separately caused mortality in the preliminary experiments or in previous releases.

The results of the acclimation for both species show that acclimation did not increase the residence time within the study area. It is unknown if acclimation increased long term survival since no acclimated specimen was detected after 4 months. These results are in contrast with those obtained by Jonssonn *et al* (1999) who had higher residence for acclimated brown trout, *Salmo trutta* and with the results of Fairchild *et al* (2010) who registered similar results for winter flounder *Pseudopleuronectes americanus.*

Comparing the results for the acclimated fish only, it is interesting to note that one *D. sargus* returned to the study area on several occasions even after being in the wild for 3 months, entering the study area by it's NE extremity. The fact that even hatchery reared fish (with no previous knowledge of the area) return to this area was observed in the previous study (Lino et al, 2009). But the same also happened for Ds-NA1 and for a much longer period, so acclimation did not seem to have any added value.

The Weighted Residence Index showed that although there were no statistically significant differences between the residence times of the two species or between the two treatments, the residence time of the non-acclimated white seabream is considerably higher than any of the other groups, as can be observed from the chronogram.

The white seabream has a high fidelity to his home habitat. This was demonstrated by the results obtained in the previous study (Lino et al, 2009) and also by a study using only wild caught white seabream in the Gulf of Castellamare, Italy (D'Anna et al, 2011). In this study the authors also proved that *D. sargus* has a clear homing behavior which could explain why released fish return to the release site.

The Weighted residence Index seems to be a more indicative measure of fish residence. It does not give excessive importance to fish that stay in the area for consecutive days and it is more robust to periods of non-detection due to difficulties in receiver replacement. As an example, fish Dc-NA3 which only was detected during the day of release has an I_R (sensu Afonso, 2008) of 1 (meaning always resident) and an I_{RW} of 0.00001 (since it is weighted for the whole study duration).

The DTI value seems to be a good measure of the fish activity and can be calculated with only two points which is an advantage over the MCP. The DTI values show that fish that use the same MCP area can have different levels of activity, moving frequently within the area. As an example it also shows that fish Ds-A2 in spite of only being detected on 7 days, moved around extensively covering nearly 80% of the total MCP area.

The last detected position shows no pattern either per species or per treatment. However it seems to indicate that most of the fish followed the prevailing current direction and moved towards NW. The fact that the last detection for 3 fish was at a

central position could indicate that they were fished since this is a location frequently used by the artisanal fleet (Santos, pers. observation)

An interesting observation about the two acclimated *D. cervinus* is that although one of the fish remained within the study area and the other was not detected for days, the last detection for both was on the same day, on the same receiver, so it is a possibility that they schooled, which would be an interesting result for restocking actions.

In conclusion the use of acclimation cages did not increase site fidelity. Although 'life skills training' for hatchery fishes (Brown & Laland, 2003) such as acclimation is important this was not a successful option. On the other hand if it was, then the next step would be scaling up, which as mentioned by Huntingford (2004) would be a challenging task. Further studies are needed to investigate other methods aiming to increase site fidelity. These could include creating feeding stations which would function as a temporary food source and then slowly wean off the fish. Another option would be to increase the complexity of reefs with refuges that the fish are previously adapted to in the Aquaculture Station.

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Table 1 - Characteristics of the tagged *Diplodus sargus* and *Diplodus cervinus* specimens. TL is the Total Length; FL is the Fork Length; TW is the Total Weight; and RI is the Residence Index.

ID	Species	Treatment	TL (cm)	FL (cm)	TW (g)	Days detected	Number of detections	IR	I_{RW}	Average I_{RW}
Ds-NA1	*D. sargus*	Non-acclimated	23.8	21.3	241	8	2513	0.05634	0.01481	0.198
Ds-NA2	*D. sargus*	Non-acclimated	23.1	21.3	230	147	77213	0.87500	0.32186	
Ds-NA3	*D. sargus*	Non-acclimated	23.5	21.0	264	132	134471	0.88591	0.25633	
Ds-A1	*D. sargus*	Acclimated	23.2	20.9	234	6	699	1	0.00047	0.004
Ds-A2	*D. sargus*	Acclimated	23.1	20.6	222	7	197	0.07692	0.00830	
Ds-A3	*D. sargus*	Acclimated	23.7	21.2	218					
Dc-NA1	*D. cervinus*	Non-acclimated	24.1	21.5	280	13	177	0.13131	0.01677	0.013
Dc-NA2	*D. cervinus*	Non-acclimated	22.9	20.5	230	41	21583	1	0.02191	
Dc-NA3	*D. cervinus*	Non-acclimated	22.8	20.7	223	1	3	1	0.00001	
Dc-A1	*D. cervinus*	Acclimated	24.5	22.0	296	14	413	0.82353	0.00310	0.002
Dc-A2	*D. cervinus*	Acclimated	22.7	20.5	231	3	397	0.17647	0.00066	
Dc-A3	*D. cervinus*	Acclimated	24.2	21.8	276					

Table 2 - Chronogram of the detections of acoustic tagged fish. The study was carried out between August 6th 2008 to May 25th 2009 but no detections were made after January. In the fish ID field Ds = *Diplodus sargus*; Dc = *D. cervinus*; NA = Non-acclimated and A = Acclimated. The shaded areas represent days with detections.

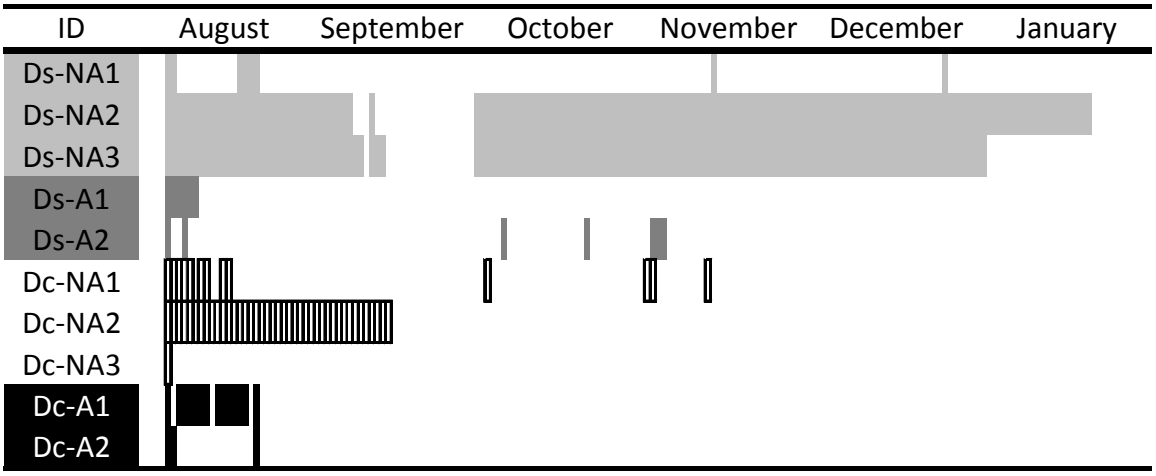

Table 3 - Habitat preference for the two species analyzed (*Diplodus cervinus* and *D. sargus*) comparing Acclimated and Non-acclimated groups. ST1 to ST15 are the passive acoustic stations

Station	*Diplodus cervinus*						*Diplodus sargus*						Total
	Acclimated			Non-acclimated			Acclimated			Non-acclimated			
	Shallow	Mid	Deep	Shallow	Mid	Deep	Shallow	Mid	Deep	Shallow	Mid	Deep	
ST1			321			59			42			1858	2280
ST2			489			6123			16			44056	50684
ST3						13						25	38
ST5					225			24					249
ST6					321								321
ST7					1898								1898
ST8					12939			39			166325		179303
ST9					91			82			209		382
ST10							39			1198			1237
ST11				1			426			111			538
ST12				20			78			43			141
ST13				2			1			115			118
ST14				71			98			167			336
ST15							55			90			145
Total	**0**	**0**	**810**	**94**	**15474**	**6195**	**697**	**145**	**58**	**1724**	**166534**	**45939**	**237670**

Table 4 – Measure of the fish activity. Ds = *Diplodus sargus*, Dc = *D. cervinus*, NA = Non-acclimated, A = Acclimated. DTI is the Distance Traveled Index. MCP is the Minimum Complex Polygon.

ID	Number of receivers	DTI (km)	MCP (km2)	% of Total MCP	Last Detected
Ds-NA1	10	13.46	4.66	65%	ST8
Ds-NA2	3	2.16	0.28	4%	ST8
Ds-NA3	4	8.79	0.78	11%	ST1
Ds-A1	8	10.59	3.27	46%	ST11
Ds-A2	7	11.12	5.67	79%	ST5
Dc-NA1	3	2.88	0.36	5%	ST1
Dc-NA2	10	35.60	4.11	58%	ST8
Dc-NA3	2	1.13	NA	NA	ST9
Dc-A1	2	2.01	NA	NA	ST1
Dc-A2	2	1.01	NA	NA	ST1

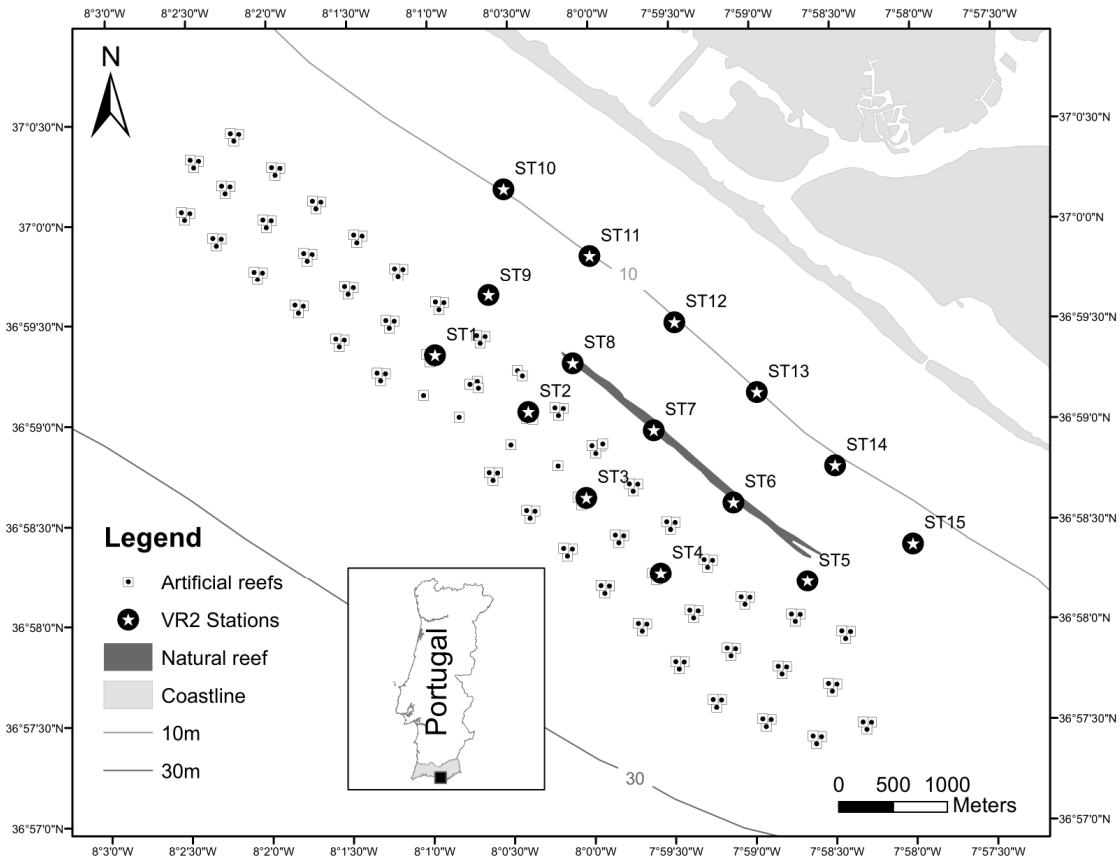

Figure 1 – Map of the study area. The 15 passive acoustic stations (ST1 to ST15) are represented by the black circles with a white star. ST1 to 4 constitute the Deep (Artificila reef) row, ST5 to 9 the Middle (Natural reef) row and ST10 ro 15 the Shallow (Sand) row

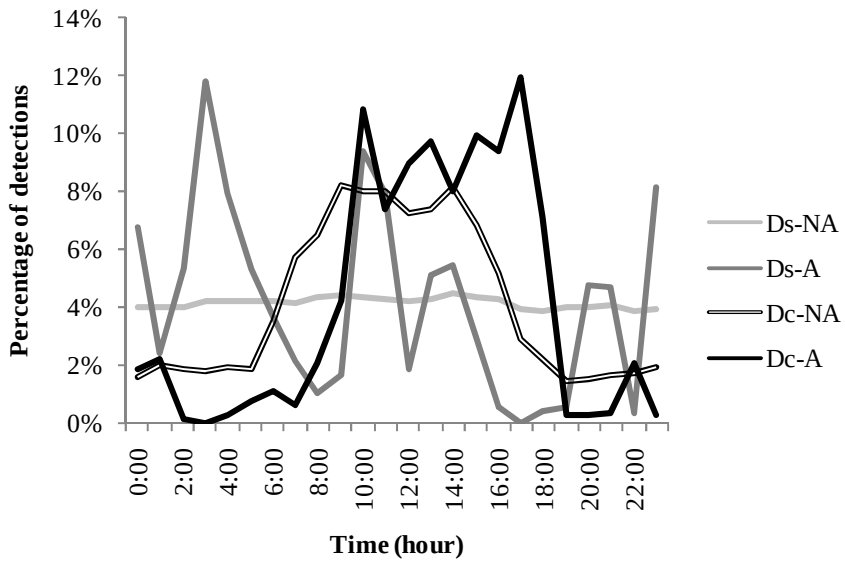

Figure 2 – Daily activity pattern for the four groups of fish. Ds = *Diplodus sargus* ; Dc = *Diplodus cervinus*; NA = Non-acclimated; A= Acclimated

CHAPTER 8

Conclusions and suggestions

The current work used several methodologies which allowed the assessment of the potential of restocking as a useful tool for contributing to the management of small scale fisheries in a local perspective.

Underwater observations are limited by dive time, light and sea conditions and they typically cover only a small part of the animal's lifetime resulting in an underestimate of the utilized area (Kerwath 2005). Underwater visual censuses are also an extremely limited tool in terms of spatial coverage. Each observation is limited by the underwater visibility. In addition the duration of the observations is limited in time by the air supply. The number of observations is also limited by the number of divers and each diver is limited by saturation in CO_2. However underwater visual censuses are the richest tool in terms of results obtained because they rely on actual direct observation. This method was therefore extremely useful for describing the behavior of fish at release time and also to compare behavior between wild and hatchery produced fish.

The initial use of conventional tagging was extremely important. It is a "low tech" tool which requires a high initial effort with a lot of manpower hours in catching, anesthetizing and tagging of fish, but it has a low equipment cost which allows for massive tagging of large numbers of fish. The fact that no active effort is required by this method to recapture fish is both an advantage and a disadvantage: the majority of the costs can be allocated to producing the fish with a smaller proportion for advertising and rewards. The obvious disadvantage is that effort in recapture is not managed and therefore it is not evenly distributed or easy to assess. The area covered by the network of potential collaborators is much larger both in space (at least the whole South coast of the Algarve) and time (depends only on appropriate fishing time for the species released) than any research institute could afford to cover. The success of the returned

results depends essentially on the advertising and on the good relationship with the fishing community. Since in the particular case of this study the species tagged are exploited both by the professional fishermen as well as the recreational, it involves a relatively high effort in advertising but covers a high number of potential collaborators.

The use of smaller than legal size tagged *D. vulgaris* and *D. sargus* in the VIE experiment (Lino *et al*, unpublished) showed that even if the relationship with the fishing community is good, the level of trust is not high enough to report illegal sized catches. In addition, the fact that fish were released inside a local lagoon where the use of fishing gears that could be used to catch fish as small as those released is illegal (e.g. fine mesh beach seines and beam trawl) also contributed to the absence of reported captures. These results were not unexpected since Erzini *et al* (2002) also faced the same near absence of returned fish even after tagging thousands of under sized wild fish all year long.

The quality of the returned data from conventional tagging varied greatly from a simple "I captured fish number X at the Faro pier last month" to fish actually returned intact with a precise GPS position. However, the current study also confirmed that the amount of returned fish is only a fraction of those captured. Most fish were not returned because of the size (under MLS) or because of the capture location. But many were not returned simply because fishermen did not bother to call the phone number displayed in the tag. Even fishermen who initially returned fish, as time went by stopped doing so because they already had collected all type of rewards. Although it was not possible to test this hypothesis it is the author's belief that a monetary reward would have yielded higher return rates. However the value of the reward would have to be weighted in order to avoid promoting an increased effort to capture tagged fish. The modification in the

reward amount (high reward- low reward method) would also allow the estimation of the proportion of unreported captured fish (Pollock et al 2001). In spite of the low results of the tagging with VIE experiment, it proved to be an interesting method to apply to Sparids. It is a non-lethal, inexpensive method that allowed tagging specimens below the MLS (e.g. for *Diplodus sargus* as small as 6cm in Total Lenght) where a T-bar anchor tag would certainly have some impact on the swimming performance. In addition it allows to easily separate between batches using different colors. However because the tag is not easily identifiable by professional or recreational fishermen it requires a lot of effort and expenses from the research institution when used in a wild habitat.

Conversely conventional marking and releasing fish with T-bar anchor tags provided long term results over an extensive area. Although the majority of the reported fish were caught off the South coast of the Algarve, one fish was reported as far East as the Bay of Cadiz and another as far North as the Basque country. Curiously no fish were reported from the Portuguese west coast further North than Sines. Most of the fish were captured within a month of release but returns extended in time up to more than two years which indicates a longer term effect of restocking with the selected species.

Acoustic telemetry is an expensive tool which can return an impressive amount of information if the researcher has the equipment to make the adequate experimental design. In terms of spatial coverage it is not as wide as conventional tagging but it is several orders of magnitude superior to underwater visual censuses. In terms of temporal coverage it is currently the best possible tool that can be used for studying the underwater behavior of fish. It monitors and stores data 24 hours / 7 days a week. If the tagged fish is within the range of one or several receivers the presence is registered and

associated with a known location. Conversely the absence of detection is also a result. In addition to the fact that passive acoustic telemetry is not limited by visibility (although detection range may vary due to acoustic noise) it is also not affected by the amount of available light thus making it the perfect tool for night time movement detection (in contrast to visual censuses which are either not possible during the night time or require a source of artificial light which will influence behavior)

The results obtained with acoustic telemetry on the movements of *Diplodus sargus* show that the interpretation of the visual census was largely correct. Diving and counting fish on the same reef group indicated that tagged fish remained for less than 30 days at release location. However acoustic telemetry demonstrated that although they may not remain in the same reef group (and therefore could not be detected by subsequent dives) they may remain resident within the reef (artificial and natural) area for over six months.

The current work also tested if acclimating fish for a few days in a cage positioned at release depth could increase site fidelity as observed for other species (Jonssonn, Brannas & Lundqvist 1999; Kuwada et al 2000; Brennan, Darcy & Leber 2006). Unfortunately for the species used the results showed that acclimatizing does not increase site fidelity. Although this was an unexpected result, the opposite would also be of little practical advantage if the experiment was upscaled. Placing cages underwater to house the millions of fish required for a real restocking action would be unfeasible.

The current study also demonstrated different results with species even from the same genus. While results for *Diplodus sargus* were most satisfying, results for *Diplodus cervinus* were less successful. Even for extremely related species such as *D. sargus* and

D.vulgaris which are commonly associated in the wild, the results obtained with experiments carried out (unpublished data) showed that handling of *D. vulgaris* caused extreme scale loss and mortality even before tagging. This means that even if this species was an important resource to be restocked it would be extremely difficult to evaluate stocking success due to the difficulty in tagging. Obviously new methods such as genetic markers based on detected genetic variations (Feral, 2002) might be a future solution for such species but currently the cost of running genetic tests to separate wild from released fish is currently still not realistic.

The analysis of the genetic diversity of two of the species produced in the IPIMAR aquaculture station demonstrated that although some diversity was lost in comparison to the wild populations, there were no signs of inbreeding or depression effects, which means that proper hatchery management of the brood stocks used for restocking is being carried out. These results were not surprising since IPIMAR is a research institute where 20% of the brood stock is replaced annually with new wild specimens. Since all brood stock is composed of wild fish, all fish produced are first generation in captivity which means that there is no inbreeding. The slight loss of genetic variation detected is simply caused by the reduced number of fish in the brood stock, compared to those in the wild population.

Finally, the results of the fish returned showed that over time fish were in good condition and that only 11 days after release, the stomach contents of released *D. sargus* included brachyuran crabs (not locally used as bait) thus indicating that they were already actively capturing live food. The observed increase in body weight of *D. cervinus* after release after the initial loss is in agreement with the adaptation to natural food. The fact that the fish condition factor is lower than the before release is probably

in agreement with the standard condition factor of wild fish and not an indicator of under feeding.

All the above results indicate that Sparids, namely *Sparus aurata*, *Diplodus sargus* and *D. cervinus* are good candidates for restocking actions. The results also show that releasing hatchery produced fish that lived in shallow tanks at depth is not a good option. Even though large adult *Diplodus sargus* use the artificial reefs as a breeding location (Leitão and Santos, 2009) juvenile fish do not find it suitable as a permanent habitat. In fact similar sized wild *D. sargus* captured in the nearby natural reef use the artificial reefs during daytime (possibly as a feeding location or as refuge) but prefer the natural reef during the night time.

One the major goals of the establishment of a restocking program is to reduce costs since these actions are mostly funded by public institutions (although as mentioned previously, they should involve the fishing and aquaculture industry and common funds). In that respect the fish used for restocking actions should be as young as possible in order to reduce production costs. Unfortunately this study did not return results in terms of the smallest size that could be used since no results were returned when under-sized fish were released in the wild.

Nevertheless as mentioned before, restocking actions only make sense when it can be established that the cause of stock depletion has been removed (e.g. by modifying the gears responsible for the catches of the juvenile fish) and that the cause of stock depletion was not a reduction in the carrying capacity of the habitat.

Therefore further studies on this subject could follow several lines of research:

* an ecosystem wide, multi-disciplinary approach to evaluate the carrying capacity of

the Algarve coastal waters for the species selected;

* using other economically important species of other families (e.g. *Dicentrarchus labrax* or more sedentary species such as the dusky grouper *Epinephelus marginatus*);

* investigate other tagging methods such as genetic markers, chemical tags or food induced modifications which would remove the minimum size for tagging limit and provide inter-generational tags;

* establishing protected areas to restock and compare with simultaneous restocking actions in exploited areas

CHAPTER 9

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