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Escape-related behavior of the main Mediterranean farmed species - gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*)

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ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
ΣΧΟΛΗ ΘΕΤΙΚΩΝ ΚΑΙ ΤΕΧΝΟΛΟΓΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
ΤΜΗΜΑ ΒΙΟΛΟΓΙΑΣ

Μελέτη της συμπεριφοράς διαφυγής των κυρίαρχων εκτρεφόμενων ψαριών στη Μεσογειακή Υδατοκαλλιέργεια – Τσιπούρα (*Sparus aurata*) και Λαβράκι (*Dicentrarchus labrax*)

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Σας ευχαριστώ όλους θερμά!!

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Abstract

Greece, Spain, Italy, Turkey and France are the major contributors to the total Mediterranean and European aquaculture. The main farmed species in the Mediterranean region are the gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), while particularly in Greece their species make up for 95% of the total national fish production. With the recent tremendous growth of the Mediterranean fish industry during the last two decades, fish escape from sea cage facilities increases the pressure upon a forthcoming sustainable industry.

Fish escapes have been reported for almost all the European farmed species, including sea bream and sea bass. Fish escapes disclose a considerable socio-economic effect to the relevant countries, with additional disclosed ecological and genetic impacts on the marine environment. As a considerable indirect effect from fish escape is the reduction on the fame of the industry and of course the loss of money and also several conflicts with groups related with the protection of the environment. Additionally, as the last stage, consumers might pay more for a fish that has been mistakenly considered as fisheries. Further, interaction with the wild stocks, interbreeding, food and mate competition and transfer of pathogen and parasites are major examples of serious environment interactions. The main cause of fish escape is a combination of structural failure of equipment with severe environmental conditions, such as strong winds and storms. Predators and pelagic animals attacking the offshore farm facilities are also another cause for fish escape. Recently, species-specific behavioral attributes have been also correlated with a potential risk of escape. Indeed, fish farmers have widely documented interactions towards the net pen, concerning net inspection and bite, mostly regarding Atlantic cod (*Gadus morhua*) and sea bream. These interactions, also called “pre-escape” behavior disclose a potential risk of net damage and a creation of a hole that will eventually lead to fish escape. However, knowledge of the related factors, both biotic and abiotic, that affect sea bream and bass escape behavior as well as the relative net interactions that potentially lead to net damage is almost nonexistent until now.

The main objective of this thesis was to provide basic knowledge regarding the related factors that affect the escape behavior of the main Mediterranean farmed species. In addition, species-specific attributes that lure farmed fish to interact with

the cage net, create holes on the surface and escape are discussed. Pre-escape behavior is further evaluated in accordance with the condition of the net pen, based on different nets commonly found in farm facilities. Simulated small-scale experiments in laboratory conditions were performed and specially developed video processing and image analysis systems were used so as to evaluate species-specific behaviors in aquaculture. Moreover, preventive measures and implementations to avoid large-scale events are discussed.

In chapters 2 to 4, the potential influence on sea bream pre- and escape behavior of factors like the feeding condition of fish, applied fish density, and the presence of micro-fouling are evaluated. Sea bream usually approaches the net pen at a close distance (< 2 cm), looking for a net tear and escape. Along with escape activity, sea bream has also been reported to inspect and bite the net. The potential risk for escape is further increased by sea bream attraction towards fairly damaged nets, consisting of cut twines and fouling filaments. It is clearly demonstrated that sea bream escape behavior is strongly related with the amount of food provided and to the applied fish density. In addition, pre-escape behavior is mostly associated with the net structure, with the presence of cut twines and filaments on the net surface further induces net biting and eventually net damage.

The knowledge acquired in chapters 5 and 6 highlights the need to investigate the escape behavior of European sea bass from a different point of view. This species has no interaction with the aquaculture net, but is yet able to escape when a net tear is present. Further, sea bass is able to locate a net tear from a longer distance than sea bream. Therefore, research should be focused on external environmental factors that could alter sea bass behavior inside the cage environment and increase the escape risk. Visual conditions at the point of escape and light level were proven to have a strong influence on the escape behavior of sea bass. Swimming activity is also affected by different light level. In addition, visible or solid obstacles and illuminated areas could mitigate the potential risk for fish escape. Lastly, the use of artificial light opens a new discussion for a more sustainable aquaculture.

The acquired information regarding sea bream and bass escape behavior highlights the need to reconsider cage technology to minimize the risk of net damage and also mitigate fish interactions that may also wear and tear the net pen. Both species are able to escape, but present totally different patterns and species-behavioral attributes. Thus, cage and net materials as well as cage location and orientation regarding sea bream and bass should be reconsidered, based on species behavioral attributes in the Mediterranean aquaculture. Better quality materials and regular control of the cage and net condition could reduce the potential risk of net damage and the creation of a hole. Further, the use of undesired materials would prevent fish from exploratory behaviors towards the net pen.

The general conclusion of the present thesis is that both sea bream and bass are able to locate net tears and escape, though several factors can influence this specific behavior as well as affect relevant behavioral traits prior to any escape event.

Study of sea bream and sea bass escape-related behavior can potentially make a significant contribution to a forthcoming sustainable Mediterranean aquaculture through mitigation of large-scale events.

Management measures should be taken in the aquaculture industry so as to eliminate the potential risk of escape. Preventing large-scale events will ensure many of the previously mentioned socio-economic effects as well as the ecological and genetic impacts to the marine environment. It is clear that with the high expansion of offshore aquaculture in the future, operational procedures and fish handling should be reconsidered and address fish escape as a major problem. These measurements should be cost effective and environmentally friendly but efficiently fit the industry purposes and general aquaculture expectations.

Γενική Περίληψη

Στη Μεσογειακή Υδατοκαλλιέργεια, τα είδη που κυριαρχούν είναι η τσιπούρα (*Sparus aurata*) και το λαβράκι (*Dicentrarchus labrax*). Η εκτροφή και των δυο αυτών ειδών, ιδιαίτερα στην Ελλάδα, φτάνει σε ποσοστό 95% επί του συνόλου της εθνικής παραγωγής. Συνολικά για την περιοχή της Μεσογείου, ο τομέας των υδατοκαλλιεργειών έχει εμφανίσει αλματώδη ανάπτυξη τις τελευταίες δεκαετίες, με χώρες όπως η Ελλάδα, η Τουρκία, η Ισπανία, η Ιταλία και η Γαλλία να συμμετέχουν ενεργά στην παραγωγή και μετέπειτα διάθεση των προϊόντων της υδατοκαλλιέργειας. Ωστόσο, ουσιώδη προβλήματα συνεχίζουν να υφίστανται, όπως για παράδειγμα οι παρατηρούμενες διαφυγές των καλλιεργούμενων ψαριών από τις μονάδες εκτροφής και πάχυνσης. Οι συνεχείς αναφορές τέτοιων περιστατικών τα τελευταία χρόνια έχουν αναδείξει τις διαφυγές ως μείζον πρόβλημα για τον κλάδο, απειλώντας παράλληλα τη βιωσιμότητα της Μεσογειακής, αλλά και γενικότερα της παγκόσμιας υδατοκαλλιέργειας.

Διαφυγές ψαριών έχουν καταγραφεί για το σύνολο σχεδόν των εκτρεφόμενων ψαριών στην Ευρώπη, συμπεριλαμβανομένου της τσιπούρας και του λαυρακιού. Οι αρνητικές επιπτώσεις μπορούν να διαχωριστούν σε: κοινωνικό-οικονομικές και περιβαλλοντικές/γενετικές. Οι πρώτες αφορούν στην αρνητική φήμη που αποκτά ο κλάδος καθώς και στη μείωση του κέρδους ως απώλεια αποθέματος, ενώ έμμεσα επιδρούν αρνητικά στο καταναλωτικό κοινό, καθώς συχνά εμφανίζουν υψηλότερη τιμή για το λόγο ότι λανθασμένα θεωρούνται ως φυσικά αλιεύματα.

Οι περιβαλλοντικές/γενετικές επιπτώσεις απασχολούν μια πολύ μεγάλη μερίδα της επιστημονικής κοινότητας καθώς αφορούν στη γενετική μίξη καλλιεργούμενων και άγριων πληθυσμών, ανταγωνισμό για εύρεση τροφής και αναπαραγωγή, καθώς και μετάδοση ασθενειών και παθογόνων μικροοργανισμών από και προς την ιχθυοκαλλιέργεια.

Η κυριότερη αιτία για τις διαφυγές των ψαριών είναι ο συνδυασμός ελαττωματικών υποδομών και εξοπλισμού με αντίξοες καιρικές συνθήκες, όπως για παράδειγμα, οι καταιγίδες και οι δυνατοί άνεμοι. Η παρουσία άγριων θηρευτών στην περιοχή των κλωβών ενισχύει ακόμα περισσότερο τον κίνδυνο διαφυγής, καθώς επιτίθενται στα ψάρια προκαλώντας παράλληλα και φθορές στα δίχτυα. Πρόσφατα, το γενετικό υπόβαθρο των ειδών και ειδικότερα τα πρότυπα συμπεριφοράς που παρατηρούνται στο φυσικό του περιβάλλον έχουν συσχετιστεί με την ικανότητα αυτών αλλά και την τάση για διαφυγές από τους ιχθυοκλωβούς. Πιο συγκεκριμένα, μεγάλος αριθμός καλλιεργητών, αναφέρει περιστατικά όπου τα εκτρεφόμενα ψάρια πλησιάζουν πολύ τα δίχτυα του ιχθυοκλωβού ή/και παρουσιάζουν επιθετική συμπεριφορά ως προς αυτά (δαγκώματα). Οι αλληλεπιδράσεις αυτές, επίσης γνωστές και ως «συμπεριφορά πριν τη διαφυγή» μπορούν εν δυνάμει να αυξήσουν περαιτέρω τον κίνδυνο, καθώς τα ψάρια δύναται να προκαλέσουν φθορές στο δίχτυ και εν τέλει, να δημιουργήσουν μια οπή διαφυγής. Η συμπεριφορά αυτή των εκτρεφόμενων ψαριών στην Μεσογειακή υδατοκαλλιέργεια καθώς και οι παράγοντες που, άμεσα ή έμμεσα την επηρεάζουν, έχουν ελάχιστα μελετηθεί μέχρι τώρα.

Ο κύριος στόχος της παρούσας Διδακτορικής διατριβής είναι η παροχή βιολογικής γνώσης σχετικά με τη συμπεριφορά διαφυγής των κυριότερων εκτρεφόμενων ιχθύων στη Μεσόγειο αλλά και τους αντίστοιχους παράγοντες που επηρεάζουν τη συμπεριφορά αυτή. Παράλληλα, γίνεται μια προσπάθεια ανάλυσης των ιδιαίτερων προτύπων συμπεριφοράς που εκδηλώνουν τα ψάρια εντός του ιχθυοκλωβού και οδηγούν σε φθορές και οπές διαφυγής μέσω δαγκωμάτων στην επιφάνεια του διχτυού. Περαιτέρω ανάλυση των αλληλεπιδράσεων αυτών γίνεται και σε συσχέτιση με την εκάστοτε κατάσταση του διχτυού του ιχθυοκλωβού, (φθαρμένο δίχτυ, ύπαρξη οπών, κλπ) που έχουν αναφερθεί στις εγκαταστάσεις εκτροφής.

Στα πειραμάτων, που πραγματοποιήθηκαν στις εγκαταστάσεις του Πανεπιστημίου Κρήτης, προσομοιώνεται το περιβάλλον του ιχθυοκλωβού, , ενώ αναπτύχθηκαν και πρωτότυπες τεχνολογίες παρατήρησης των ψαριών και επεξεργασίας εικόνας προκειμένου να αξιολογηθεί και ποσοτικοποιηθεί η συμπεριφορά των προαναφερθέντων ψαριών. Παράλληλα, εξετάζονται διάφορες διαχειριστικές πρακτικές, οι οποίες έχουν ως στόχο την πρόληψη και τον περιορισμό των διαφυγών.

Στα κεφάλαια 2 – 4, εξετάζεται η επίδραση παραγόντων που σχετίζονται με την εκτροφή των ψαριών (παροχή τροφής, ιχθυοφόρτιση, παρουσία αρχικού σταδίου βιο-επίστρωσης στο δίχτυ) διαφυγής της τσιπούρας. Η τσιπούρα είναι ένα ψάρι, το οποίο συχνά προσεγγίζει το δίχτυ του κλωβού σε μικρή απόσταση (<2cm), ψάχνοντας για οπές διαφυγής, επίσης. Σε πολλές περιπτώσεις έχουν αναφερθεί και φθορές στην επιφάνεια του διχτυού εξαιτίας των δαγκωμάτων της. Η πιθανότητα εμφάνισης μιας οπής διαφυγής λόγω δαγκωμάτων ενισχύεται ακόμα περισσότερο από την ύπαρξη κομμένων νημάτων αλλά και οργανισμών βίο-επίστρωσης (Biofouling), που αυξάνουν την προσέλκυση της τσιπούρας προς το δίχτυ. Τα αποτελέσματα της παρούσας διατριβής υποδηλώνουν την στενή σχέση της ιχθυοφόρτισης, της παρεχόμενης ποσότητας τροφής και την παρουσίας βιοεπίστρωσης στο δίχτυ στην εκδήλωση τέτοιων συμπεριφορών στην τσιπούρα. Επιπρόσθετα, καταγράφεται ο ακριβής χρόνος που απαιτείται για την εκδήλωση αντίστοιχων συμπεριφορών ενώ περιγράφονται λεπτομερώς τα επιμέρους στάδια της εκάστοτε συμπεριφοράς.

Στα κεφάλαια 5 και 6, η αντίστοιχη συμπεριφορά διαφυγής του λαυρακιού μελετάται σε σχέση με την ένταση του φωτός και τη χωροθέτηση των ιχθυοκλωβών. Το λαβράκι δεν παρουσιάζει συμπεριφορές ενασχόλησης και αλληλεπίδρασης με το δίχτυ του ιχθυοκλωβού και για το λόγο αυτό η συμπεριφορά διαφυγής του εξετάζεται υπό το πρίσμα εξωγενών παραγόντων και συνθηκών που επικρατούν στις περιοχές καλλιέργειάς του. Το είδος αυτό εμφανίζει σημαντικές διαφορές στο πρότυπο διαφυγής, καθώς είναι ικανό να εντοπίσει την οπή στο δίχτυ από μεγαλύτερη απόσταση από την τσιπούρα, αλλά και για το λόγο ότι η διαφυγή ενός ατόμου προκαλεί την αντιγραφή της συμπεριφοράς του από τα υπόλοιπα άτομα του πληθυσμού. Υπό συνθήκες διαβάθμισης της έντασης του φωτός, το λαβράκι εκδηλώνει μια έντονη προτίμηση στις φωτεινές περιοχές των δεξαμενών. Σε αντιστοιχία αυτής της συμπεριφοράς, η απρόσκοπτη φυσική φωτοπερίοδος στην περιοχή των ιχθυοκλωβών δεν πρέπει σε καμία περίπτωση να περιορίζεται. Διαφορετικά, η συνάθροιση του πληθυσμού κυρίως στα φωτεινά σημεία του ιχθυοκλωβού θα διαφοροποιήσει την ιχθυοφόρτιση, θα επηρεάσει την αξιοποίηση την τροφής, ενώ στην ύπαρξη τρύπας θα οδηγήσει τον πληθυσμό σε διαφυγή. Παράλληλα, η χωροθέτηση των κλωβών εσωτερικά άλλων κλωβών δύναται να περιορίσει την πιθανότητα διαφυγής, καθώς τα δίχτυα των γύρω κλωβών θα λειτουργούν έμμεσα ως περιφερειακά δίχτυα περιορίζοντας την οπτική αντίληψη του λαυρακιού για πιθανές τρύπες στο δίχτυ.

Τα αποτελέσματα της παρούσας διατριβής ενισχύουν την άποψη ότι και τα δυο εκτρεφόμενα είδη στη Μεσόγειο είναι ικανά να διαφύγουν μέσω οπών στα δίχτυα των ιχθυοκλωβών. Συνεπώς, η τεχνολογία και οι τεχνικές καλλιέργειας οφείλουν να αναθεωρηθούν λαμβάνοντας υπόψη όλους αυτούς τους παράγοντες που επηρεάζουν/σχετίζονται με την εκδήλωση συμπεριφοράς διαφυγής. Ιδιαίτερα, για την τσιπούρα, η χρήση πλέον ανθεκτικών διχτυών και ο συχνός έλεγχος τους μπορούν να συμβάλουν θετικά στην μείωση της πιθανότητας φθορών και αλλοίωσης της επιφάνειας του διχτυού και συνεπώς στον κίνδυνο διαφυγών. Τέλος, ανάπτυξη τεχνολογιών παρακολούθησης των ψαριών μέσα στον ιχθυοκλωβό μπορεί να συμβάλει περαιτέρω στην πρόληψη των διαφυγών, καθώς θα παρέχει συνεχώς στοιχεία της κατάστασής τους και των συμπεριφορών που εκδηλώνουν.

1. Introduction

1.1. Mediterranean Aquaculture

The first records of Mediterranean aquaculture come from the ancient years (2500 BC), in Egypt, when wild fish were confined into small ponds. Following this, both ancient Greeks and Romans had successfully practiced aquaculture of common Mediterranean species (gilthead sea bream and European sea bass) as well as marine invertebrates in lagoons. The precursor title of modern Mediterranean aquaculture, referred to as ‘‘*vallicultura*’’ was present in the region of the Adriatic Sea from 1500 AC (Ravagnan, 1992).

Modern aquaculture has undergone a dramatic development due to advances in the late 1970s, concerning reproduction, larvae culture, food and farming technology. Overcoming the main technology issues, fish production moved to large-scale hatchery systems and offshore facilities satisfying most of the optimistic expectations (Merino et al., 2012). In addition, the deficit of the fisheries landings spurred the growth of aquaculture, so as to follow the increasing demand for fish protein. Particularly in Europe, the share of production from brackish and marine waters increased from 55.6 percent in 1990 to 81.5 percent in 2010, driven by marine cage culture of Atlantic salmon and other species.

Mediterranean aquaculture, at the beginning of its growth, mostly focused on mollusc production. However, mariculture of fish contributed to a tremendous growth of the overall production during the last 40 years. Gilthead sea bream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*) are the dominant species accounting for 91% of total Mediterranean production (Figure 1). In addition, new species (dentex, meagre, sharpsnout seabream, etc.) have already been introduced in either small or large-scale production rate.

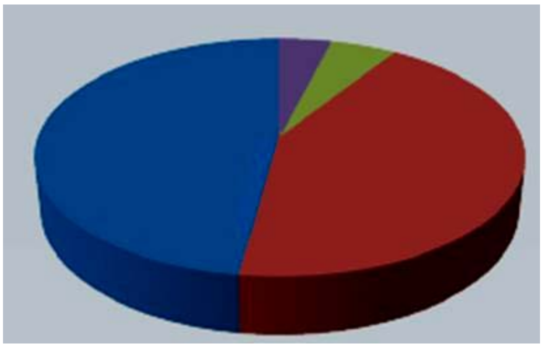

Figure 1: Mediterranean Aquaculture Production per Species in 2011. Sea bream (red), sea bass (blue), other marine species (green) and flatfish (purple). Source: FAO (2011).

The first efforts for breeding and commercial production of sea bream and bass took place in the late 1980s, focusing on controlled intensive systems in inland facilities. As the sector developed, the vast production was transferred to offshore facilities, following the technology of the mooring cages for the salmon culture in Norway (Hansen et al., 2008). This resulted into a great increase (over 300 thousand tons) of the total production for both species, where Greece, with Turkey are the leaders in the production in 2012 (FAO, 2014).

Today, Greece is the indisputable leader in the Mediterranean region (Theodorou et al., 2010) for both sea bream and bass production, with an overall 137 thousand tons, accounting for almost 40% of the global production for both species. Other countries like Norway, Spain, Italy, France and Turkey have also their significant contribution to the overall European Aquaculture (FAO, 2012). Aquaculture production is mainly dominated by fish (Greece and Turkey) and molluscs (Spain) of high economic value.

Nowadays, the Mediterranean mariculture presents a remarkable production of marine and fresh water fish that contributes to the general socio-economic structure with thousands of employees in many countries. Indeed, investments moved from the big cities to uninhabited regions and islands, contributing to the general advance of the entire Mediterranean region. Remarkably, aquaculture production surpasses most of the expectations, offering cheap and good quality fish protein to the market. Total production, including all categories (inland / offshore) and farmed species (freshwater / marine) has already increased to ~2 million tons in 2011. The geomorphology of South Europe region – sheltered bays, long coastline along with favorable climate and environmental conditions (Theodorou, 2002) provides a great potential for an efficient and sustainable aquaculture industry.

1.2. Common species in the Mediterranean Aquaculture

1.2.1. Gilthead sea bream

Animalia:	Animalia
Phyllum:	Chordata
Class:	Osteichthia
Order:	Perciformes
Family:	Sparidae
Genus:	Sparus
Species:	*S. aurata* (Linneaus, 1758)

Figure 2: Gilthead sea bream (*Sparus aurata*)

The gilthead sea bream (*S. aurata*) is common in the Mediterranean Sea, present along the Eastern Atlantic coasts from Great Britain to Senegal, and rare in the Black Sea. It is a euryhaline and eurythermal species, found in both marine and brackish water environments such as coastal lagoons and estuarine areas, particularly during the initial stages of its life cycle.

This species migrates in early spring towards protected coastal waters, where they can find abundant food resources and higher temperatures. In late autumn, it returns to the open sea, where the adult fish breed. In the open sea, gilthead sea bream is usually found on rocky and sea grass (*Posidonia oceanica*) meadows, but it is also frequently caught on sandy grounds. Young fish remain in relatively shallow areas (up to 30 m depth), whereas adults can reach deeper waters but generally not more than 150 m (Moretti et al., 1999). Sea bream is known as a sedentary species, found either in small aggregations or isolated in nature. It is mainly carnivorous, accessorily herbivorous and feeds on shellfish, including mussels and oysters (Pita et al., 2002). The age reported for this species, in nature, ranges from 1-4 years, but in aquaculture it can reach up to 11 years (Campillo, 1992).

This species is a protandrous hermaphrodite. Sexual maturity develops in males at 2 years of age (20-30 cm) and in females at 2-3 years (33-40 cm). Females are batch spawners that can lay 20 000-80 000 eggs per day for a period of up to 4 months. In captivity, sex reversal is conditioned by social and hormonal factors.

Sea bream aquaculture in the Mediterranean Sea

The gilthead sea bream is regularly present in the Mediterranean fish market. It was traditionally reared in coastal lagoons and saltwater ponds along the Mediterranean coast. Extensive culture systems still remain in some regions of the Mediterranean but with very low impact on the total aquaculture production. The establishment of reproduction and husbandry methods in the late 1980s allowed the domestication of sea bream aquaculture in 19 countries. Nowadays, cultured sea bream production is mainly realized into intensive farming facilities (sea cages, inland tanks) with an average stocking density of 20-100 kg m^{-3}. Intensive culture occurs in sea cages, either in sheltered or semi-protected areas or submerged cages. Young fish are subjected to the cage nets in stocking density of 10 – 15 kg m^{-3}. Under favorable conditions, the on-growing period (from 10 g) reach the market size (~500 g) in about one year. In the last 20 years (1990-2010) sea bream production has risen rapidly from approximately 4.000 up to 145.000 tons.

Greece is by far the largest producer in the EU (40% of the total production), followed by Turkey (30%), Spain (14%) and Italy (6%). The sector provides employment positions for over ten thousand people. Considerable production also occurs in Croatia as well as the southern Mediterranean countries (Cyprus, Israel, Egypt and Morocco). Approximately 100 companies are active in Greece, with a total sea bream production of 120.000 tons in 2012. However, the largest companies are only five (Figure 3), including Nireus, Selonda, Andromeda, Dias, Hellenic and Galaxidi (FEAP, 2010). The formidable position of Greece is mostly based on advanced fish farming techniques as well as that marketable size can be reached in 16-18 months, which is shorter compared to other countries in the Mediterranean region. Almost 80% of Greek bream production is exported to other countries (i.e. Italy, Spain, Sweden, etc), which along with the farmed sea bass make up the second national export product following olive oil (FAO 2012).

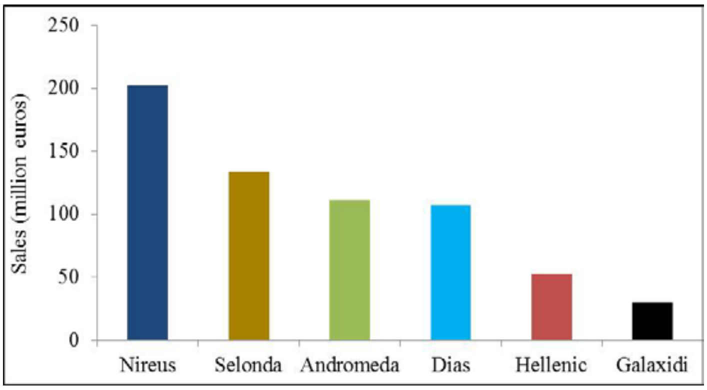

Figure 3: The largest Greek companies in sea bream and bass farming sales for 2012. *Source*: Nireus A.E.

1.2.2. European sea bass

Animalia:	Animalia
Phyllum:	Chordata
Class:	Osteichthia
Order:	Perciformes
Family:	Sparidae
Genus:	Dicentrarchus
Species:	*D. labrax* (Linneaus, 1758)

Figure 4: European sea bass (*Dicentrarchus labrax*)

The European sea bass (*D. labrax*) was the first marine non-salmonid species to be commercially cultured in Europe and is at present the most important commercial fish widely cultured in the Mediterranean region. It is the second most important species (in production volumes) in Mediterranean fish farming after sea bream.

Sea bass mostly appears in the Mediterranean Sea and the Eastern coast of Atlantic Ocean, from Norway up to Senegal. It is a eurythermic (5 – 28^{o}C) and euryhaline (fresh to sea water); thus, it inhabits coastal inshore waters down to about 100m, estuaries and lagoons. It is also possible to venture upstream into freshwater. It breeds once per year, during winter for the Mediterranean populations and up to June for the Atlantic, respectively. The preferred temperature varies between 9 - 15^{o}C, and the juvenile fish aggregate in shallow waters. Sea bass, in nature, are predators on small fish, prawns, crabs and cuttlefish. Young males become mature in 2-3 years with the females one year later. Adult sea bass can reach an age of 30 years and a maximum weight of 10 kg. Although sea bass are farmed in seawater ponds and lagoons, the vast production comes from sea cage installations in the open sea. Juveniles are subjected to as on growing stock at a size of 1.5 – 2.5 g. They reach the market size (400 – 450 g) in 18 – 24 months. During this time, fish are fed with commercial pellet every 10 – 15 minutes, every day. Grading is necessary at least two or three times per cycle, in order to avoid growth differentiation and cannibalism.

Sea bass farming in the Mediterranean Sea

European sea bass is a fish with high commercial value for both fisheries and aquaculture. Sea bass has traditionally been cultured by extensive methods in seawater ponds and lagoons. Fish then enter a lagoon, the entrance is closed and the fish are trapped inside. Trapped fish are fed naturally in the lagoon until they reach a marketable size. Intensification of sea bass production started in 1960s, but not until in the early 1980s the relevant techniques had been fully developed.

The traditional extensive culture of sea bass has recently been replaced by intensive systems, such as offshore sea cages (10-20 kg m^{-3}) or inland concrete tanks (30-80 kg m^{-3}). Sea bass farming has also shown a positive growth in the last 20 years (1990-2010) as a result of better hatching and handling processes, with an overall production of approximately 132.000 tons and an additional economic value of 786 million USD. However, major problems still exist, regarding the susceptibility to diseases, poor growth rates and sexual maturation that are mostly linked with high temperatures and faulty management practices.

Currently, sea bass farming, along with the production of sea bream makes up over 95 percent of total production in Greece. Sea bass production reached almost 120.000 tons in 2010 (FAO, 2010). Similarly to the sea bream, Greece is among the major producers with 40 percent of the total European production followed by Turkey (30%) and Spain (11%). In Greece, the total production for sea bass in 2009 was over 30.000 tons (Eurostat, 2009). Sea bass is also produced in a small scale along the entire Mediterranean region.

1.3 Cage aquaculture

Offshore aquaculture is nowadays considered as an emerging approach to mariculture Sea farms are located in deeper and less protected areas with stronger water currents than in the inshore installations. Farmed fish are stocked, artificially fed and then harvested, when they reached the market size. Sea cages were firstly introduced in the Mediterranean aquaculture in the late 1980s, since this technology provided enough space for fish production when aquaculture expanded to meet the increasing human demand for fish protein.

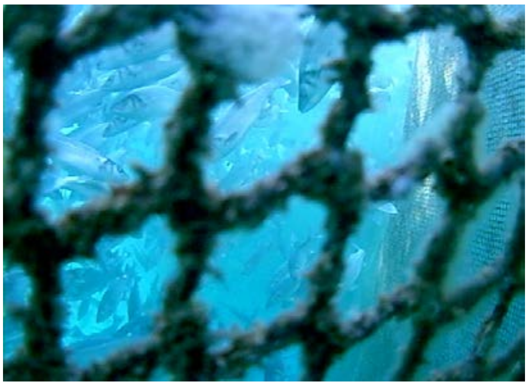

Figure 5: European sea bass inside a commercial sea cage. *Source*: Nireus A.E.

A cage with a rigid structure (more or less flexible) is supported by floats on the water surface or if submerged a few meters deeper. A net pen containing the farmed fish encloses the cage on both sides and the bottom. Mesh (hexagonal or standard diamond) can be made of different materials (nylon, polyester, polypropylene and polyethylene) but nylon (polyamide) is the most common (Figure 5).

Cages are open systems in contact with the open sea. This ensures water renewal that in turn enables water oxygenation. Food and fish waste are also removed from the cage. During culture, fish are provided with high-protein complete feed, and can live in their habitat remaining in the bounded area of the cage net.

When using sea cages, fish are able to move around with a consequent increase in appetite and growth. A greater mobility of fish due to the larger volume contributes to improved "welfare". In addition, the fish are allowed to avoid the surface level where bad weather conditions very often exist.

However, sea cages are very often exposed to severe environmental conditions, such as strong winds, waves and storms. Even at protected areas and fjords, damaged nets or entirely destroyed sea cages (Figure 6) have been reported after storms and strong winds. To improve and promote the cage-based marine aquaculture, the industry needs to address a recent problem, related to the wide-range and high-impact effect of fish escapee's from net cage aquaculture facilities.

Figure 6: A major structural damage of a cage in Norway. *Source*: Ø. Jensen.

1.4. Fish Escapes in Aquaculture

1.4.1. Definition of Fish Escape

"Fish Escape" has been defined as the loss of farmed fish through tears that exist on the cage's net (Figure 7). However, sscapes can theoretically occur at all stages of the farming process: including larvae rearing and juvenile/adult fish. The escape risk is rather low during the larvae stage, since the whole procedure takes place in closed systems. Escapes have been reported for almost all species that are cultured worldwide, including Atlantic salmon and cod, rainbow trout, artic charr, halibut, gilthead sea bream, European sea bass, meager, tilapia, and kingfish (de Azevedo-Santos et al., 2011; Naylor & Burke, 2005; Naylor et al., 2000).

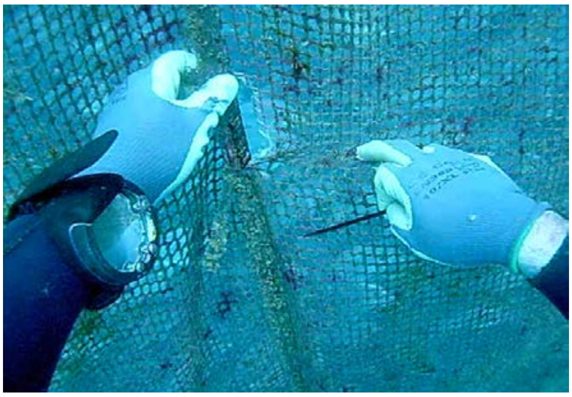

Figure 7: Diver repairs a net tear on a sea bream cage. *Source*: Nireus A.E.

Recent research has focused on a second type of escape that of viable, fertilized eggs spawned by farmed species inside the cages (Jorstad et al., 2008; Somarakis et al., 2013). Thus, the term "escape" has been redefined and now includes the escapement of the fertilized eggs into the marine environment.

1.4.2. Causes of Escapes in aquaculture

Escapes are mainly caused by a variety of incidents related to farming equipment and its operation (Jensen et al., 2010). Particularly for the European countries, causes of escape can be categorized into structural failure (52%), operational related failure (31%) and biological factors (17%). Structural failures are strongly related to severe environmental conditions (strong winds, storms and wave currents). Storm-related cages break, wear and tear of the net, and operational accidents lead to spills of fish. Specifically for the Mediterranean region, causes of fish escape (sea bream and bass) are: a) biological factors (51.5% - net biting, predator attack), (b) structural failures (39.4% - cage break, net damage, and mooring failure) and c) operational failure (6% - harvest, transport and grading).

The main cause for fish escape is structural failures of equipment generated by severe environmental conditions (Jensen et al., 2010). Farm facilities in the open sea can be damaged after strong winds, storms, waves and currents. In addition, component fatigue or human error during farm installation may also lead to escape events. Further, operational related failures including boat collision, incorrect net handling and/or net damage by boat propellers result into fish escape. Moreover, experiments identified that both sea bream (Glaropoulos et al., 2012; Papadakis et al., 2013) and Atlantic cod (Hansen et al., 2008; Moe et al., 2007) exhibit a range of behaviors that pre-dispose them to high rates of escape through holes, since they swim close to the net walls (Rillahan et al., 2011) and bite the netting. This is the main reason that sea bream and Atlantic cod present a totally different escape behavior than European sea bass and Atlantic salmon, respectively, as those species have higher probability to locate net holes on the net.

Another potential cause for fish escape is wild fish aggregation (Figure 8) and predators attacking the net cage. Birds, finfish and marine mammals are known to be attracted by floating structures in the sea, and not eaten food that falls through the cages may enhance this effect (Dempster et al., 2002). Particularly, the bluefish (*Pomatomus saltarix*) used to aggregate around sea cages and attack the cultured fish, causing serious damages to the net (Sanchez-Jerez et al., 2008). Similar problems have also been reported by bird, bottlenose dolphins and monk seals in farming facilities in Italy (Mellotti et al., 1994, 1996; (Lopez, 2012; Lopez & Shirai, 2008).

Figure 8: Wild fish aggregation outside of a commercial cage with sea bass. *Source*: Nireus A.E.

1.4.3. Fish escape: Importance and Consequences

Escape of farmed species has recently been raised as an important problem that threatens the sustainability of the aquaculture industry in Europe, and worldwide (Naylor & Burke, 2005). Regarding the economic consequences, escapes lead to direct loss of fish, while causing an extra negative publicity for the farm industry. Thus, the overall cost of escapes has its own significance for the industry in terms of lost income at the point of sale. While the economic impacts are not always evaluated, researchers from Norway assess the salmon escape to be <0.2 % of the total value of the fish held in sea cages every year(Jensen et al., 2010). Approximately 9 million escapes were reported in the period 2001 – 2005 for all European countries, according to the European project "Prevent-Escape". From the total number of 242 incidents around Europe, sea bream escapes were present in 52 compared to only 15 in sea bass. Almost 7 million sea bream were reported to escape, and in two of those incidents the number of escapees was 2 and 4 million, respectively.

Knowledge of the extent of fish escape in the European aquaculture varies significantly within countries. Some of them, like Norway, Scotland and Ireland have already established legislations to report any escape incident occurred, its extent and when it happened. Additional statistics reports (Norwegian Fisheries Directorate, 2007) allow evaluations of the problem and recommendations to minimize the risk of large-scale events. In contrast, Mediterranean aquaculture lacks statistical reports that would pinpoint the extent and the causes of escape (Dempster et al., 2009).

1.4.3.1. Environmental consequences of fish escape

Fish farming facilities are mostly located close to natural habitats, like rivers and coastal lagoons, where direct interactions between farmed and wild local populations could be present (Fleming et al., 1996; Oines & Heuch, 2007). Fish abundance inside the sea cages may reach up to hundreds of thousands of individuals with the overall number of fish held in multiple cages over a million. Due to this imbalance between caged fish and wild stocks, fish escape raises adverse concerns upon ecological and genetic impacts across Europe. It discloses a potential impact to the natural populations, since escapees increase the predation pressure on some species, such as the case of the out-migrating wild salmon smolt (Brooking et al., 2006).

The first ecological impact considers the possible alteration of the wild populations due to escaped fish from the farming facilities. Farmed fish are confined into commercial size sea cages, where they are artificially fed with food pellet. However, when they escape they are obliged to adapt to the natural environment and consume natural prey, as their wild conspecifics do.

In the case of the Atlantic salmon, several studies have already shown that this species has the same diet as wild salmon in the ocean (Jacobsen & Hansen, 2001). Similar condition factor (Hislop & Webb, 1992) and efficient adaptation to the natural environment are clear evidence of the negative consequences from salmon escape. Escaped salmon disperse rapidly and sometimes enter the rivers far from the farming locations (Thorstad et al., 1998). Similar records have been reported for Atlantic cod that, after escape, can mix with the wild populations and reach the spawning areas in the fjord environment (Uglem et al., 2008; Uglem et al., 2010). However, escapee's adaptability to the wild environment depends on the species, the age and the developmental stage of the fish (Hansen, 2006). This is further supported by several recapture efforts (Skilbrei, 2010; Skilbrei & Wennevik, 2006) that occurred very close to the farm locations and the high occurrence of artificial food on recaptured farmed species. Moreover, the limited reproductive success that has been reported for Atlantic cod and the high mortality rate of the escapees potentially reduces the risk for genetic mixture (Skjæraasen et al., 2009).

Post escape behavior of sea bream and sea bass has been recently studied (Arechavala-Lopez et al., 2013; Arechavala-Lopez et al., 2011; Arechavala-Lopez et al., 2012). Escaped sea bream have been widely reported and recaptured in the natural ecosystems of the species, and the analysis of the stomach content clearly demonstrated that this species is able to switch from artificial food to natural prey, even at the very early time of escape. The intentional release of cultured sea bream in the Southern Atlantic coast of Spain and in the Bay of Cadiz (Sanchez-Lamadrid, 2002) and in Messolonghi lagoon in Greece (Dimitriou et al., 2007) has certainly contributed to a mix of genetic stocks. Indeed, very few sea bream were found close to the farm facilities (Arechavala-Lopez et al., 2012; Dempster et al., 2002), while recaptured individuals were well adapted to the wild environment.

Only scarce information still exists on the post escape behavior of sea bass. A recent study by (Arechavala-Lopez et al., 2012)provided information on survival for prolonged periods in the open sea, although high mortality rates were also observed. Telemetry studies confirmed that sea bass is able to move quickly and repeatedly among several sea farms within the first weeks after escape and establish distinct population from the local ones (Bahri-Sfar et al., 2000; Bahri-Sfar et al., 2005). Recapture efforts demonstrated that sea bass after release mainly inhabits estuaries, where it mostly consumes natural prey. Escapes of sea bass may have detrimental effects, especially outside of the natural distribution of the species. In this case, sea bass escapees can be considered non-native species (Toledo-Guedes et al., 2012). Nevertheless, long-term ecological impacts are relatively insignificant due to high mortality levels of sea bass escapees (Arechavala-Lopez et al., 2012).

1.4.3.2. Interbreeding with the wild conspecifics

The continuous demand for higher fish production has lead into new culture practices and genetic manipulation of the farmed species, including high growth performance, resistance to diseases and adaptation to high densities. However, recent advances on fish culture disclose concerns for interbreeding between farmed and wild conspecifics and genetic alteration of wild populations.

The potential impacts from interbreeding between cultured and wild conspecifics include:

- Alteration of the genetic integrity on the natural populations due to the use of a non-local stock
- Introduction of novel and uncommon genotypes that derive from the desirable attributes in aquaculture
- Weakness of native population due to competition with the aquaculture escapees although escaped fish usually have lower reproductive fitness.

Hybridization of the Atlantic salmon discloses a number of genetic impacts on the natural population of the species. These include:

- Genetically alteration of the wild conspecifics
- Reduced local adaptation
- Affected population viability

Escaped salmon is able to disperse rapidly, enter the rivers and successfully spawn in areas with wild conspecifics (Weir et al., 2004). Hence, hybridization of farmed and wild salmons has the potential to alter the genetic viability, increase the number of alleles and consequently genetically alter the native populations (Skaala et al., 2007). Farmed salmon and hybrids are more aggressive and present faster growth in comparison with the wild partners (Thorstad et al., 1998). This gives them the opportunity to compete for food and mate with the wild fish during certain life stages. The invasion of salmon escapees in a river could eventually lead into an overall reduction in smolt production due to competition for food. When wild stocks decline, local fisheries are reduced (Fleming et al., 2000; Svasand et al., 2007). However, the potential negative influence from fish interbreeding could be restricted by lower survival rate and lifetime success of farmed and hybrids in comparison with the wild fish (Fleming et al., 2000). Atlantic cod presents a similar behavior when escaping. Also this species is able to disperse rapidly, enter the spawning areas, and hybridize with the wild stocks (Meager et al., 2010; Uglem et al., 2008; Uglem et al., 2010). Recent studies have demonstrated that also the ability of Atlantic cod to spawn in sea cages can have ecological and genetic effects. Indeed, the released eggs might enter the native spawning area, where favorable conditions could result in the survival of the escaped larvae (Jensen et al., 2010).

In the case of sea bream (Alarcon et al., 2004; Alasalvar et al., 2005; Loukovitis et al., 2012) and sea bass (Karaiskou et al., 2009), comparative studies on genetic interactions have shown no significant genetic flow between farmed and wild individuals. Nevertheless, studies have mostly been focused on escape through spawning. The prolonged time period (up to 40 months) that sea bream is now confined in farming facilities is compatible to the time needed for sexual maturation. There is evidence that during the reproduction period of the species (November-March), sex inversion occurs, and male and female gametes coexist inside the cage. Spawning within sea cages might result into an increase of the wild sea bream stocks as in the case of the Messolonghi lagoon (Dimitriou et al., 2007). Recruitment of wild stocks with spawned eggs from aquaculture might increase the fisheries catches but, the fish available to the fishery are now of much smaller size and thus, of lower economic value. Nevertheless, a comparative study (Alarcon et al., 2004) presented no evidences for genetic flow between farmed and wild fish in the same area.

1.4.3.3. Transfer of pathogens

Fish diseases and various pathogens are present in aquaculture and have sometimes a detrimental effect on the sustainability of the fish farm. Poor growth performance, high mortality and impaired welfare of fish confined in cage facilities have significant impacts in terms of production and economic performance of a fish farm. Parasites can also affect the end user of aquaculture products, since infected fish might pass the safety procedures and become available to the consumers.

From an ecological point of view, the problem becomes even bigger due to fish escapes from aquaculture. Indeed, the rapid expansion of the industry allows pathogens to freely spread into new areas. In addition, the elevated stocking densities that very often exist might also increases the concentration of the pathogen inside the cage as well the risk of spreading. Fish escapees can very often act as vectors that carry the pathogen from one farm to the other (Uglem et al., 2008).

Several studies demonstrate the potential danger to Atlantic salmon populations due to the transfer of pathogens and parasites. Particularly in Norway, salmon escapees are considered as reservoirs of sea lice (*Lepeophtheirus salmonis*), representing a significant threat to newly-migrated post-smolts and generally to the wild salmon populations (Heuch & Mo, 2001); Finstad et al., 2011; Gargan et al., 2012). (Naylor & Burke, 2005) describe the case of furunculosis, a fungal that has been transferred from Scotland to Norway and then spread to wild stocks through escapees from Norwegian farms. A huge number of salmon escapes occurred in 2007, and infected fish (salmon anemia and pancreas disease) escaped from the farms (Thorstad et al., 1998). Similar reports also exist for Atlantic cod, a species that is often coexist with the wild fish stocks, increasing the potential transmission of parasites and diseases from aquaculture (Hansen & Youngson, 2010; Oines & Heuch, 2007; Uglem et al., 2012).

Regarding Mediterranean aquaculture, there is scarce information for a potential transmission of pathogens and parasites through escaped sea bream and bass. Several important pathogens (virus, bacteria) and parasites (ectoparasites, isopoda) are documented in Raynard et al (2007). Importantly, sea bream and bass share some of the above pathogens, which increase the risk for transmission to both farmed and wild stocks. The post escape behavior of both species as documented by (Arechavala-Lopez et al., 2011; Arechavala-Lopez et al., 2012)increases the risk of pathogens transmission by fish escapes and interactions with the wild stocks. In addition, wild fish aggregation (Valle et al., 2007) around sea bream and bass farms in the Mediterranean region increases the potential transmission of pathogens, as reported in the case of grey mullet aggregating close to farm facilities in coastal habitats(Arechavala-Lopez et al., 2011).

1.4.3.4. Socio-economic effects of fish escape

The above ecological and genetic concerns about fish escape can be regarded as major concerns for a sustainable aquaculture, but the socio-economic impacts of escape are also quite important for the European countries. These can be categorized as:

- Presence of both farmed and wild fish in fisheries catches
- Detrimental effect on local fisheries with escaped fish substituting the wild catches
- Increased price for a lower quality product

Socio-economic impacts depend on the scale (small/large) of an escape event. Indeed, in the case of a small-scale event, wild stocks will not be affected in a short time, while the only possible impact could be a mislabeled escapee on the market. Customers are most affected, paying more money for a lower quality product. Consequences are more devastating in the case of a large-scale escape, where a huge number of escapees merge with the wild fisheries target. Therefore, the total income of the local fisheries becomes lower due to the lower price of the farmed fish and the significant lower amount of wild fish caught.

The influence of escaped salmon on the local fisheries has been widely investigated during the last years (Skilbrei & Wennevik, 2006; Youngson et al., 2001). Relevant studies regarding sea bream and sea bass population in the Messolonghi lagoon (Dimitriou et al., 2007) recorded an increase in the number of farmed fish in both species, probably driven by reproduction in cages and released spawn eggs. The coexistence of both farmed and wild fish caused a reduction (~ 10%) of the total income from species sale putting an extra pressure on the local fishermen.

1.5. Fish Behavior and Sustainable Aquaculture

The main objectives for a sustainable aquaculture are the production of a sufficient number of both fish larvae and juveniles, efficient rearing process until the desired age and size are reached and finally that the fish will meet the requirements of the purpose for which they were farmed. The great advance of technology has provided appropriate tools to overcome many of the issues mentioned. Thus, fish farmers are now able to increase fish production and be aware of all food and other conditions that are required (Huntingford et al., 2006).

Recently, there has been much discussion whether knowledge about animal behavior can contribute to an even more sustainable aquaculture. Understanding the mechanisms that control behavior is clearly important for aquaculture in order to provide farmers with essential information to predict and control their stocks and prevent serious problems. But what is actually meant by "Fish Behavior"?

With the term behavior, we refer to the actions and movements of an animal in response to any stimuli in the habitat it lives. It is widely believed that fishes behave as they do in order to interact and adapt to their environment and its changes. These responses are very often accompanied by physiological changes, but the key visible response is behavior. Study of fish behavior is nowadays considered as an efficient tool to overcome problems and promote sustainable development.

- One of the main reasons is that many problems in fish farming are related to behavioral patterns that fish display in their nature habitat.
- Further, through behavior in concert with physiology an organism interacts and adapts to its environment.
- In addition, fish, as marine organisms, are quite sensitive to water quality changes, and changes in behavior are their first responses to unnatural conditions. Thus, such can be considered an efficient biological indicator for low quality of water.

Several differences exist between wild stocks and farmed species in aquaculture. Indeed, the inherited cage technology from salmon farming (Hansen et al., 2008) that is widely used nowadays creates a dynamic and multifactorial environment (Johansson et al., 2006) to which fish have to adapt. Elevated stocking density and the feeding regime are important factors that induce species-specific behavioral traits in aquaculture.

Generally, stimuli are evoked by change, either in the water profile or by chemical/visual cues from for instance food. A given behavior is performed following exposure to a combination of stimuli. Coping behavior (Koolhaas et al., 1999) could explain the response of farmed fish to new challenges in the cage environment. In that way, behavioral studies can help us to understand the individual’s capacity to cope with the aquaculture rearing process.

1.6. Objectives of the present PhD Thesis

The lack of knowledge of the biological parameters and key factors that may induce fish escape-related behaviors in commercial-scale aquaculture restricts researchers and fish farmers from implementing measures and actions aimed to reduce or prevent from large-scale events. Determined efforts should thus be undertaken to mitigate the escape risk.

The objective of this study is to assess the main husbandry factors that could increase the propensity of both sea bream and bass to escape from sea cages in the Mediterranean aquaculture. The research was focusing on the above biotic and abiotic factors that could potentially influence escape behavior. Better understanding of the conditions that induce species motivation to cross an existing net tear is of great importance for helping both farmers and researchers to develop mitigation measures so as to ensure low escape risk. In addition, the behavior repertoires of the two species including net inspection, overt net biting that can result in structural weakness of the net and exploratory behavior to cross a net tear was examined. Lastly, time-dependent analysis of specific behavioral patterns was performed with the use of custom-made computer-vision system to describe the discrete steps of bite and escape pattern inside the cage environment.

1.6.1. Critical factors influencing escape rate

Several factors can potentially affect the escape rate in commercial-scale aquaculture, while many of them can hardly change, whereas other can. Such examples could be the proportion of food supplied to fish population inside the cages, the applied fish density, and the general environmental conditions around the cage, the light conditions and the development of biofouling. Feeding behavior is assumed to have a great influence on fish behavior under captivity, while it is involved in interactions with the net wall in relation with the hunger status of the confined fish. Similarly, the applied stocking density could influence fish activity inside the cage, with further consequences on school structure, vertical distribution and exploit of the cage volume. Lastly, an external factor with relevant significance on the escape risk could also be the development and therefore presence of biofouling on the cage net, which thereby creates an attraction and motivates fish to exist very close to the net pen. Of course, the aforementioned factors are strongly related with the specific behavior of the farmed species and this is the main reason that has been only investigated in Gilthead sea bream. Instead, the absence of any net interaction in the case of European sea bass along with totally different behavior under captivity turned our research on distinct external factors related to the light level and the outside environment.

The species have been selected based on their significance on the Greek fisheries and aquaculture market. Particularly for sea bream, behavioral studies have been also focused on fish interactions (inspection/nibble/bite) towards the net pen, behavior better called as "pre-escape". It is widely believed that sea bream interacting with the net surface express innate behavior patterns probably related to prey search or schooling. Particularly for these species that exhibit a propensity to inspect and bite the cage wall, research should be focused on a) the motivation behind these behaviors, b) the factors that can increase the risk of these behaviors occurring, and c) the measures that can be taken to reduce or militate against these risks.

1.6.2. Specific objectives

The present thesis is mainly aimed at:

- Controlled tank experiments that investigated the influence of the following factors on the escape-related behavior of sea bream and sea bass: the applied fish density, the amount of food provided, the presence of micro-fouling on the net pen and the light levels. In addition, three net conditions (normal, fairly damaged net and net with a tear), commonly found in aquaculture were tested in relation to sea bream interaction with the net.

- Controlled sea cage experiments that examined the effect of the immediate surroundings on the escape behavior of sea bass. Visible and solid obstacles were used to evaluate the effect of sea bass propensity to cross the net hole.

- Time-dependent analysis of species behavioral patterns of pre-escape and escape behavior through monitoring fish activity, video processing, and analysis of the relevant sequence of frames. Differences in the escape pattern will provide essential information regarding farmed species under captivity.

- Identifying the behaviors and situations that may pre-dispose fish to escape is a key step in designing farming strategies to reduce escape risks. Such strategies could for instance be to keep well-fed fish in appropriate stocking density in sufficient light.

2. Escape-related behavior and coping ability of sea bream due to food supply

2.1. Abstract

Escape of fish is a significant problem for the sustainability of the aquaculture industry. In the Mediterranean Sea, sea bream (*Sparus aurata*) has been reported to escape from sea cage installations. However, the related factors leading to this behavior still remain unknown. This study focuses on the impact that the food supplied to sea bream has on its interaction with the net and therefore its escape behavior. We test three food ratios (2, 1 and 0 % of the total initial body weight; FC2, FC1 and FC0 populations, respectively) and three different conditions of the net (flawless mesh, mesh with a cut twine and mesh with a tear) that are commonly found in aquaculture. Data acquisition and analysis are carried out with a tailor-made system developed in our laboratory for this purpose. We demonstrate that food supply is associated with fish interactions and the aquaculture net, while net condition is also an important stimulus for sea bream inspection and biting the net. Limited-fed fish (FC1) are more prone to interact with the net, which consequently leads them to either bite net wears or escape through tears.

2.2. Introduction

Mediterranean countries are the major contributors in European aquaculture production (FAO 2010). The main species that are produced are the Gilthead sea bream *Sparus aurata* and the European sea bass *Dicentrarchus labrax*. Both species are economically important, particularly for Greece, as together they constitute 95 % of the country's total aquaculture production (FAO 2009).

Technical and operational innovations have significantly restricted the number of economic and environmental impacts caused by the intensification of the aquaculture industry. Nevertheless, considering the EU aquaculture, a large number of escape incidents have been reported by insurance companies and fish farmers (EU FP-6 ECASA360 project; http://www.ecasa.org.uk). In the Mediterranean Sea, no significant advance has been made concerning this serious problem, while important points strongly connected with the escape-related behavior of farmed species still remain unstudied (Dempster et al., 2002). On the other hand, salmon and cod escape behavior is quite well documented Norwegian Fisheries Directorate 2007; (Hansen et al., 2008; Jensen et al., 2010; Moe et al., 2007). Furthermore, Norway introduced in 2009 a technical standard that gives the technical rules and regulations implemented to all Norwegian fish farm facilities, in order to increase the efficiency of fish farms and reduce the number of escapes (NS9415, Standards Norway 2009).

Escapes may have significant ecosystem impacts (Naylor et al., 2000; Uglem et al., 2008). Transfer of pathogens from the reared fish, inbreeding and competition for resources are some of the main negative effects (McGinnity et al., 2003), while various studies have shown that farmed cod enter the wild populations and disperse rapidly, overlap with wild populations and end up in their spawning grounds (Uglem et al., 2008). Large-scale escapes are caused by structural failures of equipment and severe environmental conditions, such as strong winds and storms. Furthermore, species-specific behavior is strongly related to escapes, in the way that fish are interacting with cages through biting resulting in the creation of holes (Hansen et al., 2008; Jensen et al., 2010; Moe et al., 2007). Particularly for sea bream, as reported by farmers, the damage of the aquaculture net is correlated with the existence of strong jaws. The strong anatomic mouth characteristic assists sea bream in damaging the net (Figure 9a, b).

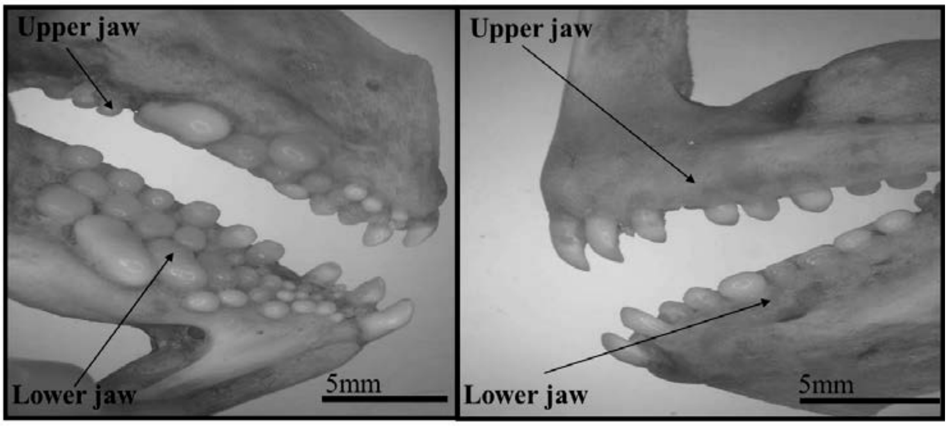

Figure 9: Morphology of sea bream’s jaw a) internal and b) external.

Predators attacking the net cages are another potential cause for escaping. Seals, sea-lions (Johnston, 2002) as well as pelagic fish (Dempster et al., 2002) are known to be attracted by floating structures in the sea, while the unused food that falls through the sea cages may enhance this attractiveness.

As related to the rearing conditions, sea bream shows direct competition for food and social interactions when held in tanks (Montero et al., 2009). A possible explanation for this is the behavioral changes associated with energy conservation and intensified prey search, which are also examples of condition-related effects of food deprivation (Skajaa and Browman 2007). Furthermore, increased activity is a behavioral reaction to food stimuli particularly when the nutritional state is reduced (Yacoob and Brownman 2007; Skajaa and Brownman 2007;(Hansen et al., 2008).

Several biotic and abiotic factors have been associated with the escape behavior of sea bream, even though basic knowledge is still missing (Dempster et al. 2007). Farmed fish are exposed to a dynamic and multifactorial environment (Johansson et al., 2006) that induces them to adapt through coping behavior. Coping behavior is defined as a coherent set of behavioral stress responses, consistent with time as well as common for a certain group of individuals (Koolhaas et al., 1999). Further examination of the mechanisms and factors, which underlie on individuals capacity to cope in aquaculture environment, is still missing to assist with the prevention of large-scale events.

In this work, the relation between the food supply and the escape behavior of sea bream is examined. Furthermore, behavioral traits like fish inspection and net biting are evaluated under various net conditions commonly encountered in aquaculture.

2.3. Materials and methods

This study was carried out at the aquaculture laboratory facilities of the University of Crete. The effect of the amount of the provided food on the behavioral variation in sea bream was examined, under controlled small-scale experiments.

2.3.1. Tanks preparation

Experiments were performed in nine parallelogram tanks (length 115 cm, depth 40 cm, width 34 cm). The internal surface of the tanks was painted white. One of the longest sides of the tank was replaced by a thick transparent glass allowing fish observation. Each tank was filled with 100 l of salt water. Light was provided for 12 h / 24 h (08.00 a.m. to 20.00 p.m.) by two 30W fluorescent tubes positioned 30 cm from the upper side of the tanks (Figure

Figure 10: Aquaculture Laboratory facilities at the University of Crete.

10). Each tank was connected to an external biological-mechanical filter (EHEIM 20 W).

The water temperature was set to 24^{o}C, the salinity was 38 ppt, and the oxygen saturation was above 85 %, throughout the whole experimental period. The concentrations of nitrite and ammonia were 0.3 and 1.5 mg l^{-1}, respectively. Tanks were oriented in a vertical plane array formation of 3 by 3. Each column of the array represented a triplicate for statistical analysis, while each triplicate was differentiated by the food supplied to fish.

2.3.2. Experimental fish

A total of 405 (9 x 135) sea bream, of approximately the same weight and length (Table 1), were used in this study. Sea bream individuals were provided by the Institute of Aquaculture of the Hellenic Centre for Marine Research. Larval rearing until metamorphosis was performed with the technology of mesocosm (Divanach & Kentouri, 2000) in 40 m^3 tanks. After that, fish were transferred to a 10 m^3 pre-growing tank, for 150 days. At the beginning of each experiment, a selection process took place, during which fish of approximately the same size and weight were collected and separated into 9 groups. Fish groups were placed into 20 l plastic bags and transferred to the University of Crete, where they were placed into the experimental tanks (15 individuals/tank or 6.5 – 7 kg m^{-3}).

Table 1: Initial fish measurements (mean weight ± SD in grams and average total length mean value ± SD in centimeters), total initial body weight (in grams) as also duration (in days) of the experimental sets (n=3). Exp. A, B and C refer to flawless mess; mesh with a cut twine; and mesh with a tear respectively.

	Exp. A	**Exp. B**	**Exp. C**
Duration (days)	21	21	21
Mean Weight (g)	30.34 ± 1.7	31.25 ± 1.47	30.93 ± 1.88
Average Total Length (cm)	13.17 ± 0.33	13.49 ± 0.44	13.61 ± 0.44
Total initial body weight (g)	455.22 ± 5.21	460.9 ± 5.39	461.15 ± 3.47

2.3.3. Computer vision system

The observation of fish population in the tanks was achieved by nine color digital CCD cameras (Fire-i, Unibrain), each one recording a single tank. Each of the camera’s requested frame rate was set to 9 frames per second, allowing for a smooth observation of fish movements. Acquisition was performed by an advanced version of a computer vision system, particularly developed for the experiments (Papadakis et al., 2012). The cameras were recording continuously from 8.00 until 20.00 o‘clock daily, for the entire duration of each experiment. Remote, real-time observations of fish behavior in all tanks were also achieved by the use of a web-server (LabView, Web Publishing Tool) installed together with the acquisition software. Thus, the influence of human presence on fish behavior was minimal, while the observation of the progress of the experiment proceeded.

2.3.4. Experimental design

Sea bream individuals were left to acclimatize for a period of 3 days, where they were fed with 2 % of their total initial body weight, once every day. After the acclimatization period, the tanks were split into two areas (60 and 40 % of the volume) by a removable mesh (white aquaculture net, 17-mm hexagonal mesh opening) fixed in a plastic frame (31 cm x 28 cm) that was fitted tightly to the bottom and the two sides of the tank. Fish were confined to the larger area of the tank (left side), referred to as the ‘‘holding’’ area, while the smaller one was referred to as the ‘‘escape’’ area (Figure 11). Food was always provided at the same point in each tank, at the left corner of the ‘‘holding’’ area. During the experiments, fish were fed once every day (at 14.00 h) by hand with commercial sea bream pellets (Biomar INICIO Plus No 1,9) (58 % crude protein / 18 % crude lipids).

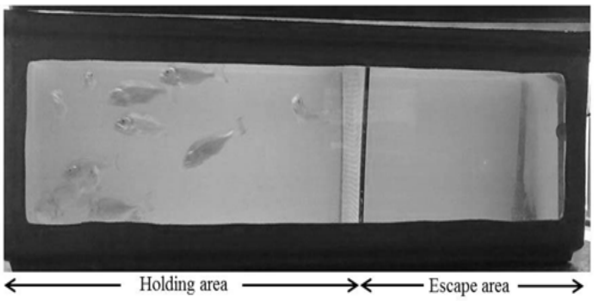

Figure 11: Confined sea bream in the holding area of the tank

The study consisted of three successive experiments; each one performed with different fish subpopulations extracted from the same initial population. The biological parameter under examination was the feeding condition (FC) of each group of fish. Three feeding conditions were tested in triplicates: 2 % (FC2: well-fed fish), 1 % (FC1: limited-fed fish) and 0 % (FC0: fish under starvation) of the initial body weight of all fish individuals in the tank. During the first experiment (Exp. A), the removable mesh was flawless in all the tanks. In the second experiment (Exp. B), one cut twine was located centrally on the mesh (fairly damaged mesh). In the third experiment (Exp. C), the removable mesh had three adjacent cut twines, forming a vertical tear (4 – 4.5 cm) located on the middle of the mesh. Through the tear, fish could cross and swim to the escape area. Equally, the escapee could also return to the holding area. In order to form a tear of the appropriate size, the height of the fish was measured (4 ± 0.12 cm) from the beginning of the dorsal fin.

Each experiment lasted 21 days. Fish ‘‘condition’’ and welfare were evaluated with respect to the classical growth performance parameters, measuring the weight and the total length prior to and at the end of each experiment. In detail, the specific growth rate (SGR = 100 x (lnWf - lnWi) / T), the feed conversion ratio (FCR = WTFS/(Wf-Wi)) and the initial (CFi) and final (CFf) condition factors (CF = W/L^3 x 100) were calculated (Table 2), where W is the body weight; Wi and Wf are the initial and final values (g); WTFS is the total dry food supplied (g); L is the total body length (cm); and T is the duration of the experiment in days.

2.3.5. Data analysis

All acquired video data were analyzed with the use of custom-made software, written in LabView (National Instruments). A time period of 15 min for each experimental day (21 days total) was selected so as to analyze fish interaction with the aquaculture net. This time period was set to 14:00 h, right before the feeding time. This duration was considered as representative to a full day period as it is comparable to related studies (Papadakis et al., 2012), where behavioral alterations remained below our detection capabilities. This study clearly demonstrates that escape-related behavior presented no significant differences ($P < 0.05$) during each experimental day.

Analysis was performed for all tanks at the same time period. Taking into account that the escape risk is even higher when farmed fish interact with the net through inspection and biting, we tested the escape-related behavior not only on clear (flawless) and fairly damaged net (cut twine) but also on net with an already existing tear. Particularly, inspection referred to the time that fish individuals spent close to the removable mesh (<2 cm), without touching it, while fish biting referred to the exact number of incidents that fish actually bite the removable mesh (Exp. A, B and C) or particularly the cut twine (Exp. B and C).

The measurement of these traits concerned (a) the number of frames per day and per tank that fish inspected the mesh and (b) the number of bite incidents per day per tank occurred on the net (Exp. A) or on the cut twine (Exp. B and C). In addition, the description of the escape behavior pattern was succeeded through the analysis of the whole duration of the acquired video data (12 h when lights were switched on). The escape and return activity were measured for all tanks, referring to the number of crossings occurred. In all the cases, the measurements were performed through the observation of the activity frames that were automatically extracted by the software, whenever a fish crossing was detected. Furthermore, the exact time of the first escape and return was recorded.

2.3.6. Statistical analysis

The fish were not individually tagged or recognizable (tracked). The statistical analysis was performed using one-way and two-way ANOVA (SIGMASTAT statistical package; Systat Software, San Jose, Calif.).

The statistical analysis of fish inspection and biting the net was performed through two - way ANOVA. Experimental days and also feeding condition (FC) were kept fixed variables, while fish inspection and biting (any place on the mesh and cut twine) were set as the dependent variables. An additional two-way ANOVA was performed, where experiments and feeding condition were kept fixed variables, while inspection and biting were set as the dependent variables. The measurements of the escape and return incidents were analyzed by two-way ANOVA. Experimental day and feeding condition were kept as fixed variables, while escapes and returns were set as dependent variables. Finally, a one-way ANOVA was performed, referring to the mean value of crossings (escape and return) for the three feeding conditions. In detail, feeding condition was set as fixed variable, while the number of crossings was set as the dependent variable.

When the data followed a normal distribution, the differences between the groups were detected using the Student–Newman–Keuls test; when the data did not follow a normal distribution, nonparametric control Kruskal–Wallis and Mann–Whitney tests were applied. Data were treated group wise ($n = 3$). The level of significance was set to 5 % ($P < 0.05$). In all the mean values, the standard error was calculated.

2.4. Results

There was no observed incident of mortality or cannibalism, during the experiments, in any feeding condition. Fish activity consisted of alternating interactions toward the net panel, such as inspection, biting, and escape. Fish inspection and biting were observed in all three experiments. In particular, fish biting on the Exp. B and C was further classified into biting the cut twine and biting any other place on the mesh. The escape-related behavior was observed only in Exp. C.

2.4.1. Pattern description of fish activities toward the mesh

Further analysis allowed the identification of two specific patterns for these activities: Biting the cut twine consisted of violent pulling and tearing movements, under successive posture (body positions) readjustments. In detail, the fish firstly accelerated while moving in the water column toward the mesh. Getting closer to the net, at a distance of around 2 cm, the fish located the cut twine and initiated biting it while bending its body to the right. Little after the first bite, the fish started pulling the twine while alters its body posture by bending to the left. Changes in body posture were a recurrent activity where the fish continued biting the cut twine. The fish completed its activity bending always to the right from the net pen. Finally, it left the area turning in any direction (Figure 12).

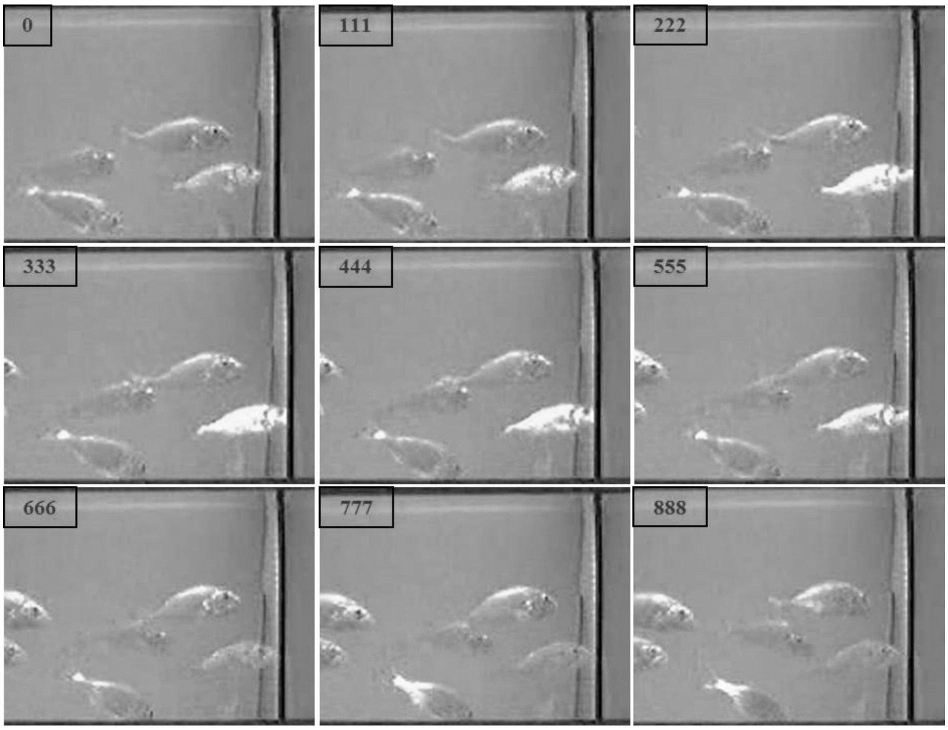

Figure 12: Time-dependent analysis of the discrete steps, regarding the bite pattern of sea bream. Frame acquisition was always accurate at 111msec.

On the Exp. C, fish had the opportunity to cross through the tear. In an attempt to initiate the crossing, sea bream always approached the tear in a vertical position to the mesh. Crossings are initiated at a distance of about 2 cm from the tear. Just before crossing, the fish accelerated and then moved toward the tear. During the crossing, the body posture remained at a vertical position until the completion of the activity and the presence of the fish at the other side of the aquaculture net (Figure 13)

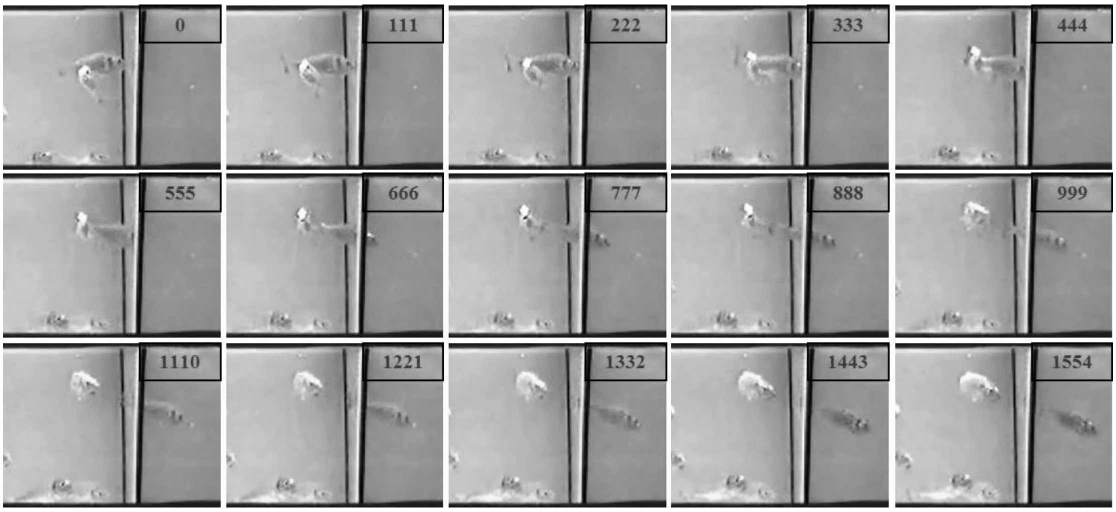

Figure 13: Time-dependent analysis of the discrete steps, regarding the escape pattern of sea bream. Frame acquisition was always accurate at 111msec.

2.4.2. Fish inspection and biting related to food supply

The expression of the two fish activities is associated with sea breams' coping behavior while the amount of food supplied and the condition of the mesh are related to the frequency of observations. In this study, a total number of 434 bites were recorded over the total experimental period and in all the experimental tanks, during the analyzed time of 15 min/day.

More specifically: In Exp. A, a statistically significant difference was observed on fish inspection between the FC2 and FC0 (Table 2). Particularly, well-fed fish tended to spend more time inspecting the net pen (42 % of their time). Additionally, the number of bites on the mesh was significantly higher on the FC2 (90 %) as compared to the FC0.

In Exp. B, it was found that sea bream were more willing to bite the cut twine (58 %) than bite at any other place on the mesh (42 %), under limited food conditions (FC1). Additionally, limited-fed fish spent a significantly higher amount of time ($P < 0.05$) on biting the cut twine than those fish belonging to the remaining two feeding conditions (Table 2). Furthermore, the inspection of the mesh was the most frequently observed activity (about 45 % of their time), even though no statistical difference was appeared between the three imposed feeding conditions. In Exp. C, the total number of bites decreased as compared to Exp. A and B (Table 2). Fish biting the cut twine showed no difference between the three feeding conditions. However, limited-fed fish bit the cut twine more than the remaining two conditions. Furthermore, escapees belonging to FC2 and FC1 were inspecting the mesh, causing a continuous attraction of fish individuals that remained in the holding area. This increased the amount of time spent on mesh inspection by (5 %) as compared to that of Exp. B for FC2 and (5, 3 %) as compared to that of Exp. A. and Exp. B, respectively, for FC1 (Table 2).

The number of bites at any place on the mesh but the cut twine was significantly higher in the flawless mesh (Exp. A) as compared to the rest two net conditions (Exp. B and C).

Table 2**:** Fish interactions with the net in the three experimental sets. The mean values ± standard errors were calculated for each triplicate, and then for the total duration of the experiment. Statistically significant differences (P<0.05) among the different feeding conditions in each experiment are presented by different letters (a, b, and c). The total number of bites is calculated from the summary of all tanks and all experimental days.

	Exp. A			**Exp. B**			**Exp. C**		
Feeding condition (%)	**FC2**	**FC1**	**FC0**	**FC2**	**FC1**	**FC0**	**FC2**	**FC1**	**FC0**
Fish inspection	160±17.45 **a**	120.66±16.79 **a,b**	97.33±23.23 **c**	123.28 ±19.33	122.95 ±13.05	163.84 ±23.9	149.68 ±24.92	125.3± 18.88	110.23 ±18.33
Fish biting the net but the cut twine	1.36±0.22 **a**	1.08±0.16 **a**	0,7±0.18 **b**	0.39±0.06	0.44±0.07	0.31±0.07	0.36±0.08	0.28±0.06	0.26±0.07
Fish biting the cut twine	-	-	-	0.22±0.05 **a**	0.61±0.12 **b**	0.23±0.05 **a**	0.23±0.06	0.35±0.08	0.26±0.07
Total number of bites	82	70	39	37	67	35	36	36	32

Significant differences were observed in all feeding conditions. The condition of the net strongly affected the number of bites at any place on the mesh but the cut twine, regardless the amount of food provided to fish individuals (Figure 14a). On the contrary, only in the limited-fed fish group did the condition of the net strongly affect the motivation of fish biting the cut twine (Figure 14b). No statistically significant differences were found between the remaining two feeding conditions. Time-dependent analysis of fish biting the net (bites at any place of the net and/or the cut twine) among the experimental days showed no significant difference ($P < 0.05$) for all the three feeding conditions.

2.4.3. Escape behavior related to food supply

In Exp. C, a total number of 432 escapes were counted among the three feeding conditions, followed by an equal number of returns. The first escape took place in the third tank of FC1 on the 5th day of the experiment. Twenty minutes later took place the first return in the same tank. On the 1st day of the experiment (1–16), there were no significant variations in the escape–return activity. Nevertheless, most of the escape and return events occurred during the latter phases of the experiment, particularly on days 17–19 and mainly in tanks belonging to FC1 (Figure 15a, b). The

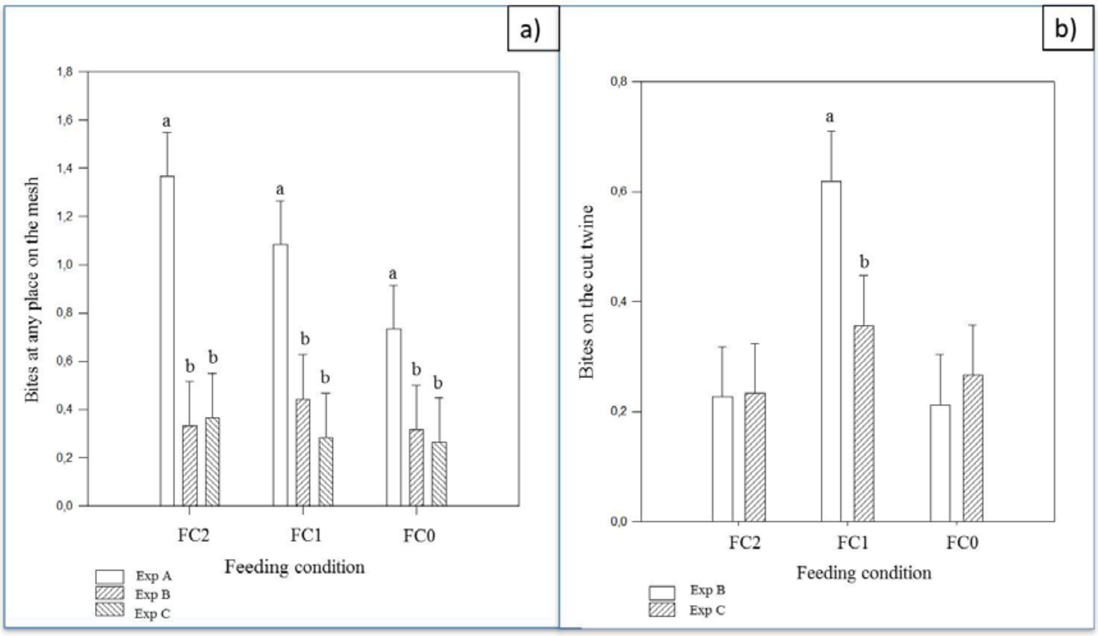

Figure 14: Number of bites occurred at any other place on the mesh but the cut twine in the Exp A, B and C. b) Number of bites occurred on the cut twine in the Exp B and C. Data are presented as mean value ± SE of each triplet of the three feeding conditions for all the experimental days. Significant differences between the feeding conditions are indicated by different superscripts (a, b).

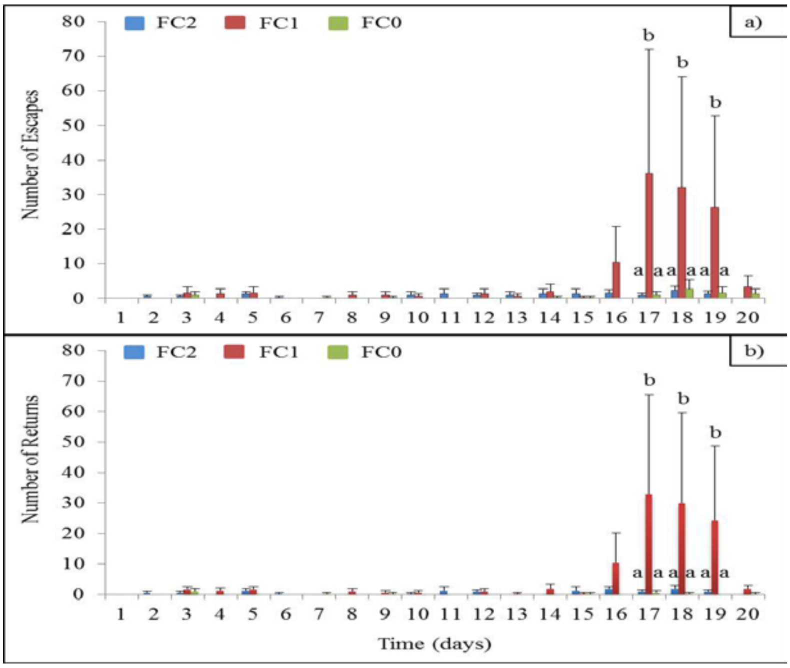

Figure 15: Number of crossings through the tear in the Exp C. Data are presented as the mean value ± SE of each triplicate, for the three feeding conditions. Each feeding condition is indicated by different column. (a) Evolution of escape incidents, (b) Evolution of return incidents.

frequency of escape-related behavior of sea bream varied significantly (P < 0.05) between the three feeding conditions. Specifically, limited fed fish (FC1) showed a significantly higher number of crossings (85 % of the total recorded events) as compared to the rest of the feeding conditions FC2, FC0 (Figure 16). In Exp. A and B, the number of bites that occurred on the cut twine as well as on any other place of the mesh was not sufficient to cause the creation of a tear due to the short experimental period.

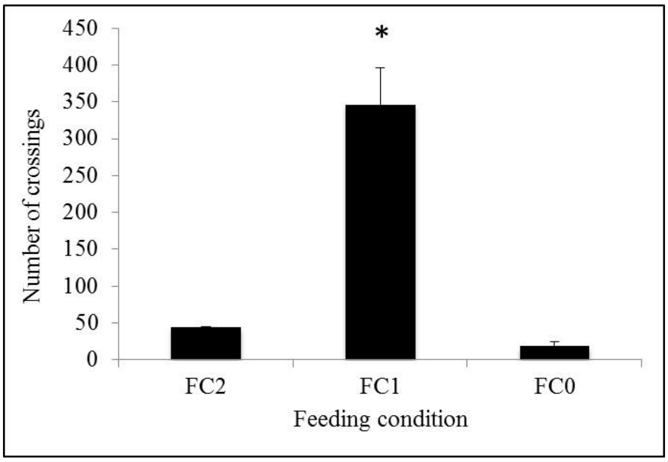

Figure 16: Number of crossings under the three feeding conditions in Exp C. Data are presented as the mean value ± SE of the total crossings (escapes and returns) of each triplicate, for the three feeding conditions, during the experiment.

2.4.4. Growth performance

Growth performance appeared to be associated with the amount of food provided to fish groups. The detailed analysis of the growth parameters showed an increase in fish weight at the end of the experiments, for all those tanks where fish individuals were fed. As shown in Table 3, well-fed fish (FC2) presented significantly higher SGR among the three experimental sets. Additionally, the FCR index on the FC0 has no value since no food was provided to that fish population. Moreover, the final condition factor (CFf) presented the lowest value in Exp. C, as compared to the rest of the experimental sets.

2.5. Discussion

Food deprivation (Montero et al., 2009; Pascual et al., 2003; Sangiao-Alvarellos et al., 2005) and high stocking density (Mancera et al., 2008) have been considered as the major stress factors in aquaculture. Food deprivation can result in both size-related and size-independent effects (Skajaa and Brownman 2007), as in altering the social interactions and behavioral traits of fish in aquaculture. The above statement is closely related to the rearing practices and technology that have been inherited from Atlantic salmon production (Hansen et al., 2008). This technology promotes productivity, but species-specific behavioral traits like the motivation to escape still remain under consideration. The current study is the first on the escape-related behavior of *S. aurata* subjected to three different feeding conditions. Each experiment was performed in tanks (small-scale experiments), relatively to small fish biomass. Fish size was selected according to a fixed population of 15 individuals per tank required to fulfill statistical purposes.

Table 3: Growth performance values of fish individuals. Statistically significant different (*P<0.05*) values are indicated with different letters (a, b or c) between the three feeding conditions. FCR value for the FC0 is 0 due to the fact that no food was provided. Symbol (-) refers to negative numbers.

	(FC2)	**(FC1)**	**(FC0)**
Exp. A			
SGR	1.28 ± 0.06a	0.63 ±0.05b	(-) 0.22 ± 0.06c
FCR	1.34 ± 0.06	1.49 ± 0.13	0
CF_i	1.32 ± 0.02	1.32 ± 0.02	1.34 ± 0.01
CF_f	1.39 ± 0.02b	1.37 ± 0.02b	1.25 ± 0.05a
Exp. B			
SGR	1.47 ± 0.09a	0.69 ± 0.04b	(-) 0.7 ± 0.18c
FCR	1.17 ± 0.07	1.35 ± 1.12	0
CF_i	1.26 ± 0.05	1.19 ± 0.006	1.25 ± 0.02
CF_f	1.47 ± 0.13a	1.34 ± 0.01a	1.1 ± 0.06b
Exp. C			
SGR	1.22 ± 0.01a	0.48 ± 0.11b	(-) 0.28 ± 0.01c
FCR	1.28 ± 0.17	1.13 ± 0.19	0
CF_i	1.22 ± 0.02	1.21 ± 0.02	1.24 ± 0.04
CF_f	1.38 ± 0.04a	1.3 ± 0.01b	1.19 ± 0.01c

The video acquisition system used was set to record 24 h day^{-1}. However, measurements were limited only when lights were switched on due to the lack of infrared illumination (Papadakis et al., 2012). Furthermore, the number of crossings that might have occurred during the night did not significantly influence our results. We have observed that at the time that lights were switched on, fish individuals were at the bottom of the tank, and raising their activity through time. This comes in agreement with sea bream's activity that is close related to the photoperiod. Gilthead and Red sea bream (*Pagrus major*) are two (relative order) species that present lower swimming activity and metabolic rate during the night (Angeles Esteban et al., 2003). Additionally, Red sea bream has been reported to stay near the bottom of the net pen, during the night (Kojima et al. 2006).

The results of the present study show that food supply can affect not only the growth performance but also fish behavior traits, including inspection, fish biting and escape activity. This statement is in accordance with related studies in juvenile walleye Pollock *Theragra chalcogramm* (Sogard & Olla, 1996) and juvenile cod *Gadus morhua* (Bjornsson, 1993), indicating that farmed marine species could alter their behavior under food deprivation. In particular, it could lead to an increase in the amount of time spent searching for prey, as well as in the intensity of the swimming activity. In addition, social hierarchies that have been documented in terms of competition for food may act as an important social mechanism that causes behavioral variations (Andrew et al., 2004; Karplus et al., 2000). In this study, differences were observed in fish interactions with the aquaculture net, depending on both feeding and net conditions.

2.5.1. Net biting: a behavioral trait of sea bream

Fish interactions (inspection and biting) were recorded throughout the day. Sea bream individuals did not show any time preference interacting with the net. The analysis of a full day showed that a 15min period of video recording can be used as representative, as it was also stated in a related study focused on the analysis of fish behavior (Papadakis et al., 2012). The large number of bite incidents that were counted among the three experimental sets indicated that sea bream is able to cause damage on the aquaculture net, even though the duration of the experiments was insufficient to allow the detection of mesh wear caused by biting. However, this statement could be explained considering the natural feeding habits of S. aurata. In nature, sea bream is fed by a variety of benthic organisms like decapods, sedentary polychaetes, gastropods and bivalves (Pita et al., 2002; Tancioni et al., 2003). These organisms are usually found in solid substrates where specific morphological characteristics are required to exploit them. Nevertheless, in captivity, it is thought that sea bream in captivity expresses its inspection and foraging activity on the aquaculture net.

This conclusion agrees with an associated study that presented similar behavioral traits in Atlantic cod *Gadus morhua* (Moe et al., 2007). The coping behavior of sea bream individuals could also explain the above statement, in association with various studies that underline the coping behavior of marine species under environmental challenges (Kristiansen & Ferno, 2007; Stien et al., 2007) Schulte 2008).

In Exp. A, B and C, food supply was strongly related to net inspection (Table 3). In Exp. A, fish biting was also strongly related to food supply. In Exp. B and C, the cut twine and the tear new features on the net acted as a trigger to the behavior of fish toward the net (Table 3). Limited-fed fish were more attracted by these features on the net, biting them, due to their increased foraging behavior. Correlations between fish behavior and food supply have also been observed in similar studies based on Atlantic cod (Skajaa and Brownman 2007) and sea bream (Goldan et al., 2003), where behavioral changes associated with intensified prey search, are examples of condition-related effects in food deprivation.

The high number of fish inspections that was observed in Exp. B, FC0, can be explained by the fact that fish showed slow swimming activity (in the video playback), due to starvation. This activity caused a prolonged presence of fish close to the net. Consequently, a higher number of related frames were extracted from the video recordings during the analysis. This is in accordance with previous studies (Sogard & Olla, 1996), concluding that fish under starvation present lower swimming activity inside the tank. Moreover, the extended food deprivation probably acted as a trigger for energy-conserving behavior (Koolhaas et al., 1999). Based on the above statements, it can be concluded that feeding condition strongly affects this behavior.

2.5.2. Food supply affects escape motivation of sea bream

In this study, we aimed at stimulating the real conditions in aquaculture. When fish were capable of locating a tear on the net, they were crossing from either side of the tank to the other. This crossing is related to the escape behavior of fish on the sea cage environment, either way. However, fish individuals in the field cross through a tear on the net and escape to the open sea, with the risk of not coming back (Dempster et al. 2007).

In our experiments, when a tear existed on the mesh, changes in fish behavior were observed. Sea bream showed less interest in biting the net and the cut twine as compared to Exp. A. This was probably due to the fact that fish initiated crossing through the tear. The frequency of the escape incidents was significantly higher ($P < 0.05$) in limited-fed fish (FC1). A possible explanation for this behavior is an intensified search for food of limited fed fish (Sogard & Olla, 1996). As stated, the escape rate in all feeding conditions presented no significant difference during the first 16 days of the experiment. This is in accordance with similar studies in Atlantic cod (Hansen et al., 2008) and halibut (Yacoob and Brownman 2007) concluding that a high escape rate is related to an intensified prey search, associated with an increased hunger level. As stated in these studies, a prolonged starvation is required to cause major behavioral changes increasing the motivation of fish individuals to escape.

Additionally, the escape rate presented variations, at the latter phases of the experimental sets, indicating the potential effect of food supply on this specific trait of marine species. The significantly lower number of escapes related to fish group with FC0 can be strongly associated with the absence of food supply. Furthermore, a significant difference was also observed between the number of crossings of the second tank of FC1 and the rest of the tanks in the same triplicate ($P < 0.05$). In other experiments with Atlantic cod (Hansen et al., 2008), the frequency of escapes appeared to be higher under high stocking densities, indicating that the escape-related behavior of marine species could be a combination of several factors, such as food supply and stocking density. The variations in fish behavior between tanks belonging to the same group (FC1) could be correlated with specific behavioral traits, better known as shyness and boldness. This particular behavioral trait is usually defined as willingness to, either take risks or get involved in competition for food (Brick & Jakobsson, 2002). Several studies have shown that bold fish tend to express the above behavior in higher rates than those that are referred as ‘‘shy’’ fish (Coleman & Wilson, 1998; Goldan et al., 2003; Sneddon, 2003). Consequently, the proportion of ‘‘bold’’ fish inside a fish population could affect the frequency of escapes.

2.6. Conclusions

In the recent years, rearing technology has advanced so as to solve rearing problems including fish diseases, mortality and growth, emerging fish escape as the major problem nowadays in aquaculture industry (Jensen et al., 2010). Several biotic and abiotic factors have been associated with the escape behavior of fish, even though basic understanding is still missing. In particular, our knowledge of the main factors affecting the escape-related behavior of farmed Mediterranean species (sea bass and sea bream) is almost nonexistent until now.

The present study indicates that the escape-related behavior of sea bream is strongly associated with the amount of the provided food to fish individuals. Additionally, net biting, which may increase the risk of fish escaping from cages, is a behavioral trait of sea bream, while it is strongly connected with the net structure. The presence of one or more cut twines leads sea bream to further damage the net, resulting to a creation of holes. In the case of an already existing hole, limited food supplies significantly increase the escape rate of farmed species. Furthermore, the fact that some fish presented a higher escape frequency than others raise the question to what extent other factors such as the genetic variability is associated with the willingness to escape (Hansen et al., 2008).

It should be noted that the study was carried out under small-scale laboratory conditions. Further examination under real conditions is needed to conclude on how feeding schedule might affect the innate tendency of species to escape from sea cages. Time is not associated with the escape-related behavior of sea bream, since fish inspection, biting and crossings occurred randomly throughout the day. Therefore, best practices routines and adapted technical and technological innovations should be established to avoid large-scale events in sea bream aquaculture.

3. Escape-related behavior of juvenile gilthead sea bream (*Sparus aurata*) versus rearing density in experimental conditions

3.1. Abstract

Fish escapes from sea cages through net tears have several environmental and economic impacts. The aim of this study is to examine how the rearing density and the condition of the net affect the escape-related behavior of sea bream (*Sparus aurata*). The trials are performed under controlled small-scale experiments and continuous video surveillance. Three densities (10, 15 and 20 individuals/tank) and three net conditions (a net pen with a tear, a net pen with a cut twine and a flawless net pen) are tested. Sea bream individuals initiate escapes shortly after a tear was created on the net. Altering the number of fish confined in tanks resulted into an exponential increase on the escape rate. Variations on fish interactions towards the net pen are also found and are associated with both rearing density and the condition of the net. Particularly, sea bream increases net inspection and biting in relation with the rearing density. Additionally, sea bream is further attracted by damages on the net pen, while it is capable of extending existing damages. Results presented are statistically significant and could act as recommendations to improve the rearing process protocols.

3.2. Introduction

Gilthead sea bream (*Sparus aurata*), as well as established farmed species, is emerging in southern Europe (Belias et al., 2003) as the primary species for rearing in sea cage installations.

Technical and operational innovations during the last years have led to a remarkable intensification on sea bream's production. However, escape incidents from sea cages that have been documented (EU FP6 project ECASA – www.ecasa.org.uk) also raised a number of economic and environmental concerns upon the sustainability of the aquaculture industry (Jensen et al., 2010; Naylor et al., 2000). Escapes can have adverse genetic effect on the native populations and also other ecological consequences. Interbreeding with native population of wild fish (Uglem et al., 2008), predation on wild fish (Brooking et al., 2006) and transfer of pathogens between farmed and wild stocks are some remarkable concerns for the marine environment because of aquaculture. Related studies on sea bream and sea bass support the above statements, as both species are dispersed rapidly from farms and/or migrate between farming facilities (Arechavala-Lopez et al., 2012; Dempster et al., 2002). Additionally, escapes are economically detrimental to fish farmers and companies as they represent losses of live animals and also due to the resulting negative publicity for the fish farming industry.

Large-scale escapes occur when holes appear on the netting pen of sea cages, but the source and cause of such holes still remain unknown (Dempster et al., 2008). In the Mediterranean aquaculture, a large number of compensations were made due to stock losses and damaged equipment during storms. These two factors have been considered as the major causes for fish escape (Jensen et al., 2010). Predators' attacking the sea cages is another potential cause (Sanchez-Jerez et al., 2008). Farms attract both prey and predator fish and also waste food that falls through the sea cages may enhance this attractiveness. Apart from operational and structural failures, a large number of farmed species (European sea bass, Atlantic cod, Atlantic salmon and gilthead sea bream) exhibit specific behavioral traits that can eventually lead to escape through holes on the net pen (McGinnity et al., 2003; Moe et al., 2007; Papadakis et al., 2012). Particularly, Atlantic cod (*Cadus morhua*) and sea bream bite the net pen and weaken it, an action that could lead to formation of holes. Both aquaculturists and insurance companies have acknowledged that escapes are unacceptable and have implemented a number of practices and legislations to reduce their frequency. Results were positive enough, as these incidents were drastically reduced in the case of Atlantic salmon (Norwegian technical standard NS 9415). However, the biological mechanisms and the risk factors that may promote species-specific behavioral traits in commercial aquaculture still remain unstudied.

Farmed fish experience non-natural high densities within the cages (Arechavala-Lopez et al., 2012), nowadays, which has also been related with increase in plasma cortisol (Ellis et al., 2002; Pickering & Pottinger, 1989) and differences in fish growth and welfare (Mancera et al., 2008). Related studies on sea bream (Canario et al., 1998) and rainbow trout (Holm et al., 1990) in tanks are consistent with the above argument, since rearing density affects growth rate (Lambert & Dutil, 2001) and behavior (Kristiansen et al., 2004) of these species.

The present study aims to evaluate the influence of rearing density on sea bream escape related behavior under experimental conditions. Furthermore, fish interaction that includes fish inspection and bite are also examined on three net conditions, commonly found in aquaculture.

3.3. Materials and methods

3.3.1. Experimental fish

Fish were provided by the Institute of Aquaculture of the Hellenic Centre for Marine Research (Crete, Greece). Fish were randomly selected from an initial large population that was previously reared with the technology of mesocosm (Divanach & Kentouri, 2000). A total number of 405 (3 x 135) juvenile sea bream, of approximately the same weight and length (Table 1), were selected and transferred to the University of Crete.

3.3.2. Tanks preparation

Sea bream individuals were confined into nine experimental tanks (length 115 cm, depth 40 cm, width 34 cm), filled with 100 l salt water (38 ppt). A thick transparent glass replaced one long side of each tank, allowing observation of the fish. The photoperiod was set to 12L: 12D by means of two 30 W fluorescent tubes positioned 30 cm from the upper side of the tanks. Water recycling was achieved through mechanical filters (EHEIM 20 Watt, Germany). The water temperature was set to 24ºC and the oxygen saturation was above 85%. Throughout the study, the concentrations of nitrite and ammonia were less than 0.3 mg l-1 and 1.5 mg l-1 respectively. Tanks were oriented in a vertical plane array formation of 3 by 3. Each column of the array represented a triplicate for statistical analysis, while the applied rearing density differentiated each triplicate.

3.3.3. Experimental design

Fish were left to acclimatize for three days in the tank environment, where they were fed "ad libitum" (approximately 2% of total initial weight), once every day. Following acclimatization period, all tanks were split into two areas (60% and 40% of the volume) by a removable net pen (white aquaculture net, 17 mm hexagonal mesh opening) that was fitted tightly to the bottom and the two sides of the tank. Fish were confined to the larger area of the tank (left side), referred to as the "holding" area, while the smaller area was referred to as the "escape" area. Sea bream commercial pellet was always provided by hand at the left side of the "holding" area, once every day (at 14:00 hr).

The study consisted of three successive experiments, where three sub populations were extracted from the same initial large population. Three different fish densities were tested in triplicates – NF10 (10 individuals/tank), NF15 (15 individuals/tank) and NF20 (20 individuals/tank) that represent 4,5 - 5, 6.5 - 7 and 8.5 - 9 kg/m3 respectively. In the first experiment (ExpTe), the removable net pen had three adjacent cut twines, forming a tear on the net surface. The tear was located centrally, and was large enough to allow fish to cross on the other side of the tank. In order to form a tear of the appropriate size, the height of the fish was measured (4 ± 0.12 cm) from the beginning of the dorsal fin. In the second experiment (ExpCt), one cut twine was located centrally on the net pen (fairly damaged mesh), while on the third experiment (ExpFl), the removable net pen presented no damage (flawless net).

Each experiment lasted 15 days. Evaluation of fish condition was achieved by using the standard growth indexes. Two measurements of fish weight and total length were performed; prior to and at the end of each experiment. Particularly in the ExpTe, final body weight and length were measured including fish from the holding and the escape area. In detail, the specific growth rate (SGR=100*(lnWf-lnWi)/T), the feed conversion ratio (FCR=WTFS/(Wf-Wi)) and the initial (CFi) and final (CFf) condition factors (CF=100*W/L^3) were calculated (Table 1) where W is the body weight, Wi and Wf the initial and final values (g); WTFS is the total dry food supplied (g); L is the total body length (cm) and T is the duration of the experiment in days.

3.3.4. Data Acquisition

Nine color digital CCD cameras (Fire-i, Unibrain) were placed in front of the tanks, each one recording a single tank. The cameras' acquisition rate was set to 3 frames per sec. Acquisition was achieved by a computer vision system (Papadakis et al., 2012). The cameras were recording continuously from 8.00 until 20.00 o'clock daily, for the entire duration of each experiment. Remote, real-time observations of fish behavior in all tanks were also achieved by the use of a web-server (LabView, Web Publishing Tool) installed together with the acquisition software. Thus, the influence of human presence on fish behavior was minimal.

3.3.5. Data Analysis

A full day observation of the video recording was performed into randomly selected tanks to have a complete understanding of fish activity during the day. Following, analysis was performed for all tanks and all experimental days (15 days). A time period of 15 minutes was selected for the analysis of fish behavior. This time period has been tested as representative of fish activity in tanks to a full day period (Papadakis et al., 2012). Time was randomly set at 13:00, 1 hour before feeding time. All acquired video data were analyzed with the use of the above mentioned computer vision system to test the escape and "pre-escape" behavior of sea bream.

Escape events are referred to as the crossing activity of fish individuals from the holding to the escape area of the tank. Additionally, the reverse activity that one fish goes from the escape to the holding area is referred to as a return incident.

The number of bites that occurred on the net pen in the ExpFl and ExpCt was not sufficient enough to result into a creation of a hole within the experimental period of 15 days. Therefore, the "pre-escape" behavior (fish inspection and biting the net) of sea bream was further examined. Particularly, fish inspection referred to the time that fish individuals spent close to the removable net pen (<2 cm), facing it and it was measured as number of frames. Fish biting referred to the exact number of incidents that fish actually bite the removable net pen (ExpTe, ExpCt and ExpFl) and/or particularly the cut twine (ExpTe and ExpCt). The measurement of these traits (counted for the 15mins daily) concerned (a) the number of frames per day, per tank and per individual that fish inspected the net pen, and (b) the number of bite incidents per day, per tank and per individual that occurred on the net (ExpFl) and on the cut twine (ExpCt and ExpTe).

3.3.6. Statistical Analysis

Statistical analysis of the data was performed by one and two-way ANOVA (SIGMASTAT statistical package; Systat Software, San Jose, Calif.). Separated tests were performed to analyze escape and ‘pre-escape’ behavior. No tagging and marking methods were used in the present study.

The escape activity under the three different fish densities was tested using one-way ANOVA. Fish density was kept as fixed variable, while the number of escapes was set as dependent variable.

The escape activity among the experimental days and under the three fish densities was also tested using a two-way ANOVA. In detail, experimental days and fish density were set as fixed variables, while the number of escapes was set as the dependent variable.

The "pre-escape" behavior was tested separately for fish inspection and biting the net pen among the experimental days under the three fish densities. Experimental days and also fish density were kept as fixed variables, while fish inspection and

Table 4: Biological parameters of experimental fish populations during the ExpTe, ExpCt and ExpFl (mean value ± SE). Values in the same row with different superscripts (a, b) have significant difference between them ($p<0.05$). The asterisk in the CF indicates significant difference between the initial and final condition factor in each density group ($p<0.05$). NF10: 10 individuals per tank; NF15: 15 individuals per tank; NF20: 20 individuals per tank

	10 individuals/tank **(NF10)**	15 individuals/tank **(NF15)**	20 individuals/tank **(NF20)**
ExpTe			
Initial Body Weight	30.45 ± 1.46	30.45 ± 1.46	30.45 ± 1.46
Initial Body Length	13.18 ± 0.24	13.18 ± 0.24	13.18 ± 0.24
SGR	1.463 ± 0.008^{b}	1.539 ± 0.135^{b}	1.142 ± 0.047^{a}
FCR	1.13 ± 0.023^{b}	1.089 ± 0.090^{b}	1.593 ± 0.151^{a}
CF_i	1.335 ± 0.001	1.324 ± 0.021	1.328 ± 0.009
CF_f	1.443 ± 0.023*	1.463 ± 0.061*	1.462 ± 0.033*
ExpCt			
Initial Body Weight	25.26 ± 1.96	25.26 ± 1.96	25.26 ± 1.96
Initial Body Length	12.5 ± 0.34	12.5 ± 0.34	12.5 ± 0.34
SGR	1.226 ± 0.086	1.230 ± 0.073	1.143 ± 0.147
FCR	1.248 ± 0.091	1.259 ± 0.09	1.399 ± 0.217
CF_i	1.422 ± 0.034	1.374 ± 0.005	1.404 ± 0.008
CF_f	1.543 ± 0.035*	1.547 ± 0.046*	1.544 ± 0.028*
ExpFl			
Initial Body Weight	25.35 ± 2.27	25.35 ± 2.27	25.35 ± 2.27
Initial Body Length	12.45 ± 0.44	12.45 ± 0.44	12.45 ± 0.44
SGR	1.42 ± 0.015^{a}	0.996 ± 0.076^{b}	1.05 ± 0.055^{b}
FCR	1.27 ± 0.002^{a}	1.868 ± 0.094^{b}	1.758 ± 0.057^{b}
CF_i	1.337 ± 0.41	1.326 ± 0.011	1.318 ± 0.011
CF_f	1.375 ± 0.354	1.329 ± 0.026	1.374 ± 0.016

biting (any place on the net pen and cut twine) were set as the dependent variables. An additional two-way ANOVA was performed to test the “pre-escape” behavior between the three experiments. Experiment and fish densities were kept as fixed variables, while inspection and biting were set as the dependent variables.

Normality tests were performed before each statistical analysis. When the data were following a normal distribution, the differences among the groups were detected using the Student–Newman–Keuls test. In the case that normality test failed, non-parametric control Kruskal–Wallis and Mann–Whitney tests were applied. Data were treated group-wise (n=3). The level of significance was set at 5% ($p<0.05$). In all mean values, the standard error was calculated.

3.4. Results

There was no loss of fish due to mortality or cannibalism during the experiments, in any of the applied fish density. Fish body weight was increased, clearly demonstrating the good condition status of fish during the experiments (Table 4).

3.4.1. Escape activity versus different applied rearing densities

In the ExpTe, fish were able to locate the tear on the net and cross from the holding to the escape area and reversely. A significant number of crossings were counted along the experimental period. In the present study, only these crossings from the holding to the escape area were measured.

A total number of 32 escapes (from the holding to the escape area) were counted by observation of the video recordings. This number of escapes was not absolute, since it was followed by an approximately equal number of return events that occurred mainly close to the feeding time. However, in the present study, we focus on the escape events.

Escape activity within the 15mins showed an exponential increase versus the three fish densities. The NF20 group presented strongly higher (p<0.01) propensity to escape in comparison with the remaining two groups (NF10 and NF15), where the same activity was similar (Figure 17). Additional analysis among the experimental days showed that the first two escapes occurred in tanks of the NF15 and the NF20, approximately 22 hours from the beginning of the experiment. In the first two days, escapes were only observed in tanks of the NF15 and the NF20. The NF10 group started escaping by the end of the second day. From day 1 to 6, escape activity was similar (p>0.05) between the three applied densities. In contrast, from day 7 to 12, the NF20 presented higher (p<0.05) escape activity (Figure 18). Escapes were reduced by the end of the experiment, without any difference among the three applied fish densities. Sea bream individuals were distributed to both

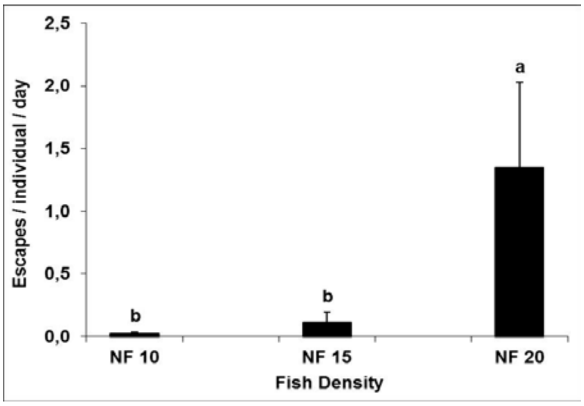

Figure 17: Total number of escapes that were counted, per individual, per day for the three density groups. Data are presented as the mean ± se of each triplicate. Statistically significant differences are indicated by different letters upon the bars (a, b). The level of significance was set to p<0.05.

the holding and the escape area of the tanks, while they were returning to the holding area especially during feeding time.

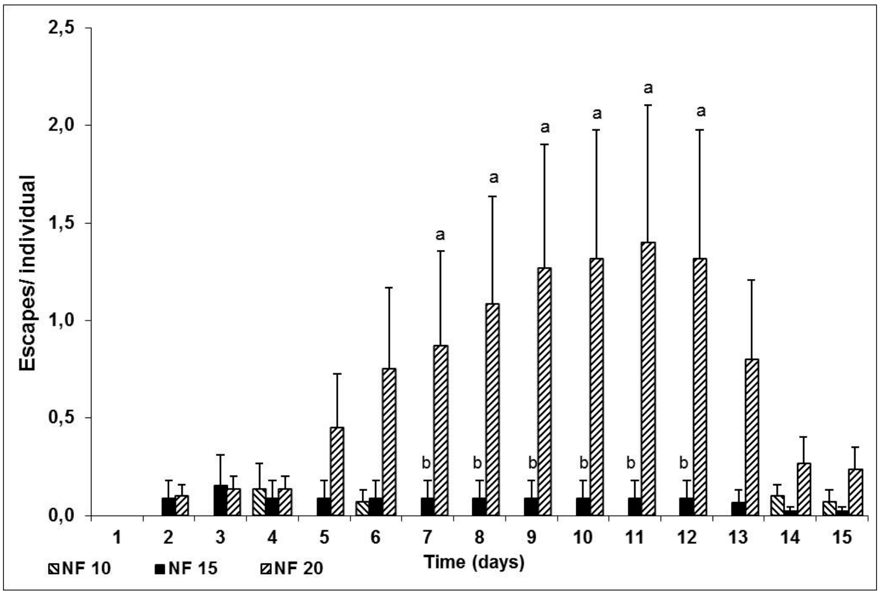

Figure 18: Number of escapes per individual among the experimental days. Data are presented as mean ± se. Each fish density is indicated by different column. Statistically significant differences are indicated by different letters upon the bars (a, b). The level of significance was set to $p<0.05$.

In the following two experiments (ExpCt and ExpFl), the absence of any net tear prevent fish from net crossing. Thus, the "pre-escape" behavior that consists of net inspection and biting was further examined

3.4.2. "Pre-escape" behavior

Sea bream activity in tanks was consisted of alternating interactions towards the net pen, such as fish inspection and biting. Both interactions (which are referred to as "pre-escape" behavior) were observed in the three experiments. Bite activity on the ExpTe and the ExpCt, further classified into; bite any place on the net pen and bite particularly the cut twine.

3.4.2.1. Fish inspection on the net pen

In the ExpTe, it was observed that the NF15 group spent more ($p<0.05$) time on net inspection. Significant differences were also observed in the ExpCt, where the NF10 group spent more ($p<0.05$) time on net inspection, while on the ExpFl, no differences existed among the three applied density groups (Table 5). The overall comparison of the three experiments showed that net inspection was related to both the applied fish density and the condition of the net. However, none of these factors presented a stronger effect on this behavioral trait.

3.4.2.2. Fish biting the net pen

In addition to the escape incidents that were observed in the ExpTe, sea bream were also biting the net pen, wearing and tearing it. The NF15 group was more ($p<0.05$) willing (≈120% more) to bite the net pen in comparison with the NF20. In contrast, the overall number of bites on the undamaged part of net pen and those that occurred particularly on the cut twine did not vary ($p>0.05$) between the density groups (Table 2).

Similar results were obtained in the ExpCt, where the NF10 group presented higher propensity to bite the net pen (≈312% more) and also the cut twine (≈271% more). Further observation of the net pens at the end of the experiment demonstrated that sea bream was able to wear and tear the net pen as well as the cut twine (Figure 19a, b).

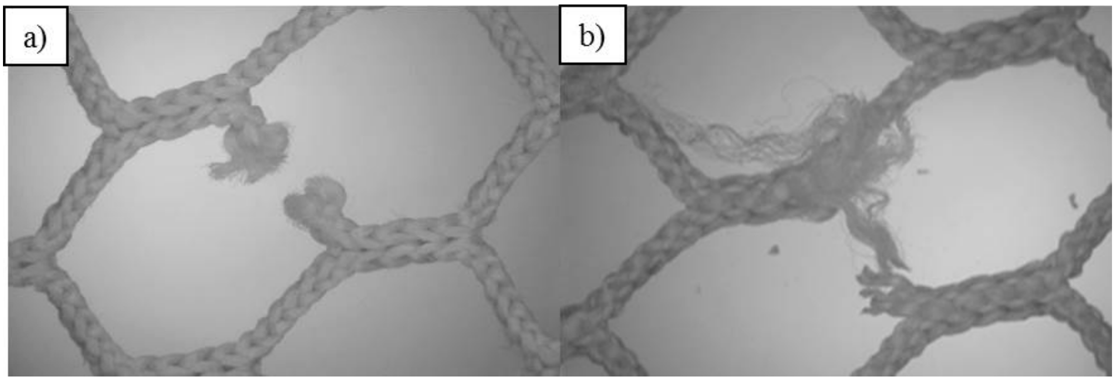

Figure 19: Photos of the net pen with a cut twine, used in the experiment. a) Start of the experiment b) End of the experiment.

In the the ExpFl, sea bream individuals nibbled the removable net pen without actually biting it. This low bite activity was also confirmed, at the end of the experiment, when the net pens were examined under stereoscope and no traces were detected. No differences were observed on fish biting the net in any of the three applied fish densities.

The comparison of the three experiments showed significant ($p<0.05$) variations on the total number of bites that occurred on the net pen as well as on the number of bites that occurred particularly on the cut twine. According to the data reported on Table 5, the NF10 were more willing to bite the net pen on the ExpCt.

Additionally, the NF10 group was more prone to bite the cut twine in the ExpCt. No significant differences were observed at the remaining two density groups among the three experiments.

Table 5: "Pre-escape" behavior (fish inspection and biting the net pen) during the ExpTe, ExpCt and ExpFl (mean value ± SE for all the experimental days). Fish inspection was measured as the number of frames per day and per individual that fish remain close to the net at a distance<2cm. Fish biting the net (any place on the net pen and the cut twine) was referred as number of incidences per day and per individual. Fish biting values the cut twine on the ExpFl is indicated as "-", represents no behavior. Significant differences among the different fish densities in each experiment are presented by different letter (a, b). The level of significance was set to p<0.05.

	ExpTe			**ExpCt**			**ExpFl**		
Fish density	**NF10**	**NF15**	**NF20**	**NF10**	**NF15**	**NF20**	**NF10**	**NF15**	**NF20**
Fish inspection/day /individual	6.1 ± 1.83**a**	12.95 ± 4.3**b**	6.05±0.77**a**	25.02±5.43**a**	7.86 ± 3.38**b**	11.57±6.16**b**	10.52±2.62	12.98 ± 6.78	11.48±2.45
Bites at any place on the net pen /day / individual	0.08 ±0.02**b**	0.11±0.02**a**	0.05±0.007**b**	1.03 ± 0.42**a**	0.25±0.06**b**	0.28 ± 0.17**b**	0.18±0.05	0.21±0.13	0.2±0.06
Bites on the cut twine/day/ individual	0.02 ±0.01	0.03±0.005	0.04±0.01	0.26 ± 0.07**a**	0.07±0.03**b**	0.07 ± 0.03**b**	"-"	"-"	"-"
All bites/day /individual	0.1±0.02	0.14±0.03	0.09±0.01	1.29±0.48**a**	0.32±0.08**b**	0.35±0.21**b**	0.18±0.05	0.21±0.13	0.2±0.06

3.5. Discussion

The present study clearly indicates that the number of fish confined in tanks is associated exponentially with the number of escapes. In all fish density groups, sea bream individuals started entering the escape area, when a tear (large enough to allow fish to cross) appeared on the net pen. Additionally, they were inspecting and biting the net pen. The total number of incidents was counted but repetitive incidents could not be identified due to the lack of fish tagging. Furthermore, the methodology used was based on video monitoring of fish population which does not allow tracking of such population without any external tagging of fish.

Comparing the ExpFl with (ExpCt), the condition of the net acted as an extra stimulus for fish attraction (pre-escape behavior). Particularly, in the ExpCt pre-escape behavior was increased while in the ExpTe, as escape activity increased, fish were less willing to inspect and/or bite the net pen.

In the ExpTe, the NF20 group presented the highest ($p<0.05$) number of escapes, indicating that an overcrowded fish population increases individual's propensity to escape. This comes in agreement with a related study, stating that the escape behavior is a combined result of several factors, such as stocking density, feeding condition and fish genotype (Hansen et al., 2008). Similarly to another study, where a prolonged food deprivation (Glaropoulos et al., 2012) is required for an exponential growth of fish escaping, a short period of time was also required in the present study for an intense crossing activity in the NF20 group. Escapes rates declined by the end of day 12 of the experiment. This change on the slope can be explained by the fact that fish distributed to the total volume of the tank, while they were entering the holding area mainly at the feeding time.

Return activity could be related to the food provided to fish population at a certain time period among the experimental days. Similar behavior has been observed in other studies (Glaropoulos et al., 2012), where returns occurred close to the feeding time.

Sea bream as well as Atlantic cod (Jensen et al., 2010; Moe et al., 2007) interact with the aquaculture net and escape through holes, while both present equal tendencies to escape at a rate that is higher than that of Atlantic salmon (Ferno et al., 1995; Juell & Westerberg, 1993).

In the Mediterranean Sea, the overwhelming majority of sea bream production is realized in sea cages and with practices that have been inherited from the Atlantic salmon production (Jensen et al., 2010). The above technology may promote high growth performance, but can also increase the escape risk even at the on-growing phase of the rearing process, where fish are still in small size. Farmed sea bream seems to express its exploring and foraging behavior on the aquaculture net pen similarly to its natural environment i.e. the bottom of the sea (Tancioni et al., 2003). Furthermore, this behavior is at a great extent associated with its structure and condition (Glaropoulos et al., 2012; Papadakis et al., 2012). Damages on the net pen that were observed in the ExpTe and the ExpCt, clearly indicate that sea bream is capable of directly damaging net material, an activity that is further induced especially at the presence of a cut twine. These damages could also be explained by the fact that the fish individuals were not genetically selected, so they may express their innate foraging behavior on the net pen. Significantly higher bite behavior in the NF10 group indicates that in low densities, sea bream might act more as an individual and not as a shoal. Thus, each individual gets closer to the net pen, inspecting and biting it. In contrast, when elevated densities are implemented, fish behavior should be considered more as shoaling behavior (Turnbull et al., 2005).

The present study was carried out under small-scale conditions in tanks and with no tagged fish. Repetitive measurements were observed probably based on the small volume of both areas and visual contact of fish individuals. Relative incidents would not be possible under different experimental design where escapees were somehow removed from the escape area or in real conditions in aquaculture. The experimental period of 15 days was not sufficient enough for fish to create a hole and eventually escape. Additionally, taking into account that each twine consists of many filaments and that sea bream individuals mean size was small, fish activity during the experimental period resulted into a gradual wear of the net pen. Future experiments in large-scale conditions, for longer experimental periods and with tagged fish could allow us to better understand sea bream's escape and pre-escape behavior.

In conclusion, the present study indicates that the higher applied fish density results into an intense fish propensity to escape. It also states that sea bream individuals interact with the aquaculture net, through inspection and/or biting, damaging it and finally creating holes. The presence of one or more cut twines (net condition) on the net is a strong factor that stimulates sea bream to further wear the net pen. A sustainable aquaculture industry has to be ethical but also environmentally and economically sustainable. Optimum settings for a future culture system in farmed sea bream could be the readjustment of the applied rearing density along with net patrolling and good quality materials.

4. Sea bream interactions towards the aquaculture net due to the presence of micro-fouling

4.1. Abstract

The technology of floating sea cages that is largely used in the Mediterranean aquaculture provides optimal conditions for the development of marine fouling. The present study investigates the influence of micro-fouling developed on a net, on sea bream behavior. Negative effects of biofouling were not tested since the study was performed under laboratory conditions and for short experimental period. The net condition (micro-fouling filaments) is further examined in comparison with a similar green-brown colored and a white net pen. From the results obtained, it can be concluded that micro-fouling filaments highly induce net biting. In contrast, the colored net tested in this study negatively affected sea bream interactions on the net. Results lead to the conclusion that the fouling filaments are more important to sea bream attraction than the net color while both variables affect species behavior.

4.2. Introduction

Aquaculture industry suffers from the presence of biofouling (Braithwaite & McEvoy, 2005; Hodson et al., 2000). Floating cages, nets and ropes serve as optimal surfaces for the development of unwanted organisms (Fitridge et al., 2012). Marine fouling is a time dependent process (Hodson et al., 1997) that initially consists of organic load, bacteria and microalgae adhesion (micro-fouling) and ends with colonization of various marine invertebrates (macro-fouling). The problem is getting even worse due to the elevated nutrient and organic load on the multi-filament netting material. In some cases, within two weeks of a cage immersion, foul organisms appeared on the net surface (Madin et al., 2010).

The nature of the negative effects of fouling has been well documented through the last years. Several problems arise in aquaculture, since fouling increases structural fatigue of cage facilities (Braithwaite & McEvoy, 2005). Particularly, it disrupts the flow of dissolved oxygen and eventually creates anoxic conditions, along with high temperature (Lai et al., 1993). Additionally, it constricts of net opening that eventually inhibits the removal of food and fish waste (Hodson & Burke, 1994). Consequently, fish well-being is indirectly affected, since farmed species are exposed into a harmful environment and they often become vulnerable to diseases. Less growth and restricted robustness are some consequences of marine fouling establishment (Fitridge et al., 2012) on sea cage installations. Lastly, economic impacts have their own importance (Hodson et al., 1997). Significant sums of money are required for net cleaning and repairing managements, contributing to a 25% cost in aquaculture (Braithwaite et al., 2007).

Marine fouling organisms have been studied extensively during the last years, but often in relation with operational problems in aquaculture (Braithwaite & McEvoy, 2005; Fitridge et al., 2012; Hodson et al., 1997). Therefore, most of the research has been directed on anti-fouling methods towards preventing the establishment of these communities. The color of the net has an influence of the settlement of algae fouling species but not on the invertebrate organisms (Hodson et al., 2000). Additionally, several colored net (red, white, black, blue, yellow) that had already been tested in a related study (Braithwaite et al., 2007; Guenther et al., 2009) had no significant difference on the fouling settlement.

However, research should also focus on behavioral variations of the farmed species due to the development and/or presence of biofouling on the cage net. Adhesion of unwanted organisms is not only considered as a supplementary food source (Moring & Moring, 1975) but it also differentiates the net surface - in color and condition. Net deformation may alter fish behavior as well as motivate fish to interact with the net.

Sea bream is among the species that is widely cultured in the Mediterranean. The vast production is mainly performed into mooring sea cages at the open sea that are largely exposed to biofouling development. Fish attraction towards the net pen may be strongly related with biofouling presence, since some studies indicate that farmed species interact with the net pen, through nibble, inspect and bite (Glaropoulos et al., 2012; Jensen et al., 2010; Papadakis et al., 2012). Particularly, for sea bream, bite activity is further increased in the presence of cut twines on the net surface. Biofouling filaments that cover the net surface are very often similar to cut twines and so, they may be related with fish attraction (Papadakis et al., 2013). Net damages can eventually result into tears and fish escape. Apart from the economic impacts, ecological concerns rise due to the interactions of the escapees with the wild populations (preventescape.eu).

The present study addresses fish behavior due to biofouling variations on the aquaculture net. The hypothesis tested is to examine whether the presence of micro-fouling or the color on the net induces fish attraction in experimental tanks. Experiments were held in specially modulated tanks and relatively designed to provide additional information about sea bream interactions with the net pen.

4.3. Material and methods

Sea bream individuals were provided by the Institute of Aquaculture of the Hellenic Centre for Marine Research (Crete). A total number of 135 juvenile fish (Table 6), of approximately the same weight (33.56 ± 0.37 g) and length (14.66 ± 0.18 cm) were randomly selected by an initial large population and sequentially allocated into nine (9 x 15 individuals) experimental groups. Following, fish were transferred at the experimental aquaculture installations at the University of Crete.

Table 6: Fish measurements: Initial and final mean body weight and length; Growth performance parameters (SGR, FCR, CFinitial and CFfinal). Data were treated group wise (n=3). Statistically significant differences between CFi and CFf are indicated by an asterix. The level of significance was set at 5% (P<0.05).

	Micro-fouling net (MN)	White net (WN)	Colored net (CN)
MWi	33,79 ± 0,38	33,49 ± 0,36	33,41 ± 0,43
MWf	44,4 ± 0,46	43,35 ± 1,04	43,36 ± 0,91
MLi	14,56 ± 0,56	14,63 ± 0,45	14,79 ± 0,23
MLf	14,82 ± 0,5	14,97 ± 0,42	14,97 ± 0,44
SGR	1,36 ± 0,06	1,29 ± 0,12	1,3 ± 0,06
FCR	0,6 ± 0,08	0,61± 0,06	0,67 ± 0,01
CFi	1,08 ± 0,03	1,09 ± 0,04	1,03 ± 0,03
CFf	1,32 ± 0,01*	1,33 ± 0,03*	1,29 ± 0,02*

4.3.1. Tank preparation

The experiment was conducted into nine oblong tanks of 100 L. Fish observations and video recording was achieved through a 5 mm thick glass, at one longest side of the tank. Water temperature into the tanks was set to 24 ºC, the salinity was 38 ‰ and the oxygen saturation was approximately 90 %. The concentration of nitrite and ammonia was respectively less than 0.27 mg/l and 1.4 mg/l. The photoperiod in the area of the tanks was set to 12 L: 12 D and it was achieved by two 30 W fluorescent tubes positioned 30 cm on the upper side of each tank.

4.3.2. Experimental design

Fish were left to acclimatize for a period of three days, while they were fed once daily (2 % of their initial body weight). Following acclimatization, the tank was split into two areas (50 %) by a removable net frame (common white polypropylene net), fitted tightly the bottom and the two sides of the tank. Fish were confined into the left area of the tank. Food was supplied at the upper left corner of the tank; daily at 14:00. Tanks were oriented in an array formation of 3 by 3, with each column of the array differentiated in net condition.

Three net conditions were tested in triplicates: a) a common white-color net (WN), b) a net with alive micro-fouling (MN), and c) a colored net, painted similar to micro-fouling (CN). The two altered conditions of white net were all developed by the same white net where: in (MN) condition a white net was left for a period of one month in a tank of HCMR with already existing biofouling, until micro-fouling filaments appeared on the net surface; and in (CN) condition a nontoxic, natural paint was used to convert the white net into a green-brown colored net. The green-brown color was chosen because it is similar to the early stage of micro-fouling in aquaculture (Figure 20a, b).

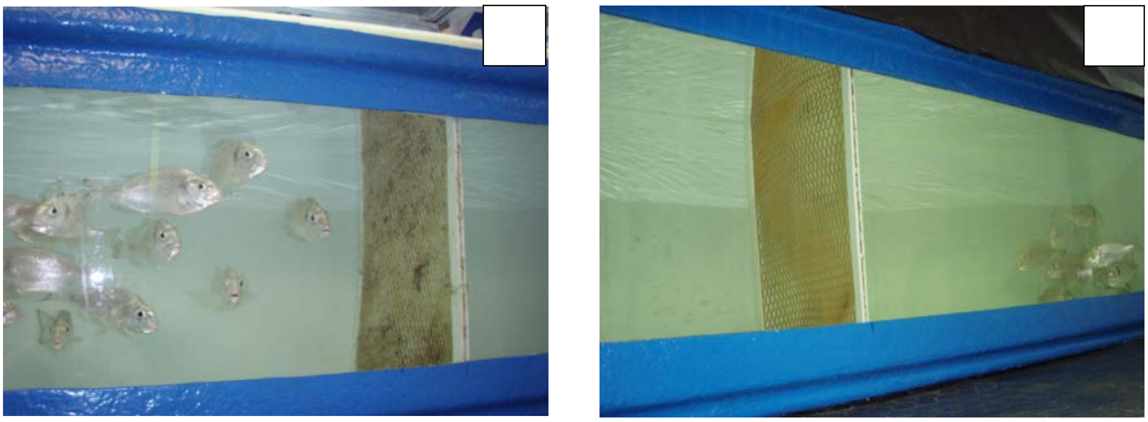

Figure 20: a) a net with alive micro-fouling (MN) and b) a colored net, painted similar to micro-fouling (CN)

Fish well-being condition was estimated at the end of the experiment, with respect to the classical growth performance parameters (Glaropoulos et al., 2012). Measurements were taken for the total length and weight prior to and at the end of the experiment (Table 1).

4.3.3. Video recording of fish activity

Behavioral monitoring of fish activity in tanks was performed by nine color digital CCD cameras (Fire-i, Unibrain) that were placed in front (<1 m) of the glass side of each tank. The requested frame was set to 9 frames per second, allowing a smooth observation of fish movement. Acquisition was performed by an advanced version of previous custom-made software (Papadakis et al., 2012). Fish were monitored continuously (8.00 up to 20.00), for the entire duration of the experiment. A remote, real-time observation of fish activity in tanks was also achieved by the use of a web-server (LabView, Web Publishing Tool), installed together with the acquisition software. Thus, the influence of human presence on fish behavior was minimal (only during the feeding time).

4.3.4. Data extraction and analysis

The acquired video recordings were analyzed with the use of custom made software, written in LabView (National Instruments), so as to detect any fish interactions towards the aquaculture net. The behavioral traits examined in this analysis were net inspection and biting. Fish inspection referred to the time that fish spent close to the net surface (<2 cm), without touching it, and the duration of this activity was measured as number of frames (9 frames = 1 second ± 111ms). Fish biting the net surface was measured as number of bite incidents.

For comparison of the aforementioned behavioral traits, data were extracted every 3 days (day 1, 4, 7, 10, 13, 16, 19) during the entire experimental period (20 days) and for five time periods within a day. Each of them had duration of 15 minutes, while they were randomly selected (08.45 – 09.00; 10.50 – 11.05; 13.35 – 13.50; 15.10 – 15.25 and 18.05 – 18.20), for statistical purposes. The same analysis was performed for all tanks and for all time periods. The overall measurements refer to the average calculation of all activity throughout the time periods within the day and for all tested days.

4.3.5. Statistical analysis

The fish were not individually tagged or recognizable (tracked). Statistical analysis was performed using one-way and two-way ANOVA (SIGMASTAT statistical package; Systat Software, San Jose, Calif.).

Two-way ANOVA analysis was performed for fish inspection and net biting throughout the experimental days. Experimental days and also net condition were kept as fixed variables, while the number of frames (net inspection) and bites (net biting) was set as the dependent variable. An additional two-way ANOVA was performed where time periods and net condition were kept as fixed variable, while the number of frames for fish inspection as well as the number of bites were set as the dependent variable.

The overall measurements were used for the comparison of the three net conditions. Finally, a one-way ANOVA was performed, where the net condition was the fixed variable and the dependent variable was the number of frames (net inspection) and bites (net biting) relatively.

When the data were following a normal distribution, the differences among the groups were detected using the Student–Newman–Keuls test; when the data did not follow a normal distribution, non-parametric control Kruskal–Wallis and Mann–Whitney tests were applied. Data were treated group wise (n=3). The level of significance was set at 5% ($P < 0.05$). In all mean values, standard deviation and error was calculated.

4.4. Results

Fish reacted immediately at the presence of the net in all tanks by alternating actions, such net inspection (<2 cm) and biting. However, fish activity on the net did not result into any apparent damage of the net. Differences in the net color and/or presence of biofouling had an influence on net interaction. The relative analysis for the overall net activity showed no differences between treatments; however the CN group had the lowest activity (Figure 21).

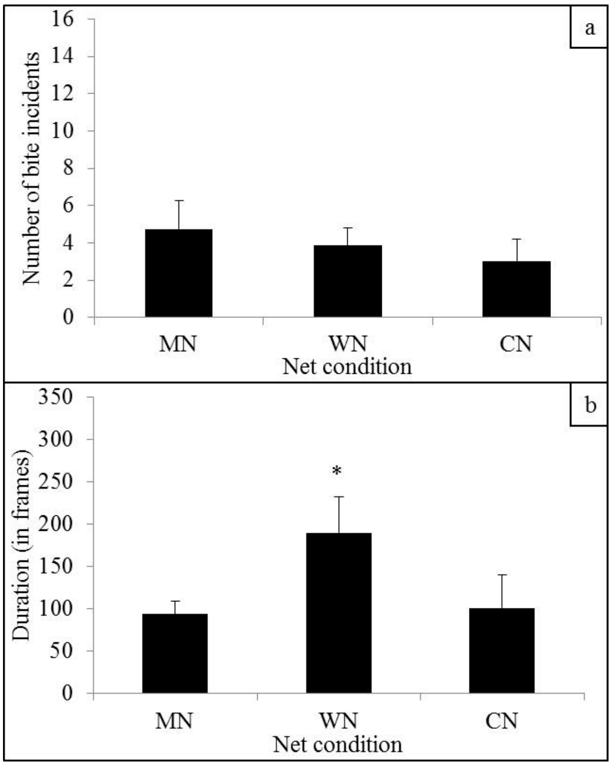

Figure 21: Overall fish activity towards the pen in the three net conditions: a) Bite activity is calculated as number of incidents, b) Duration of net inspection is calculated as number of frames. Statistically significant differences are indicated with an asterix.

4.4.1. Net biting

A high number of bite incidents was recorded during the experiment, in all tanks and net conditions. Sea bream activity towards the net on the MN group resulted into a significant reduction of the micro-fouling filaments by the end of the first day. Further analysis within treatments showed variations that were related with the net condition:

- In the first day, a strong (P < 0.001) motivation to bite the MN was observed. On average, eleven incidents were measured within the day, which was significantly higher than the related activity at the remaining days (Figure 22a).
- Bite activity on the WN group showed no significant variations. Bite incidents that occurred within the day varied from 3-6. Exceptionally on day 16, the overall bite activity within the day was found significantly lower than the relevant activity at the remaining experimental days of analysis (Figure 23a).
- Bite activity on the CN group presented a significant increase along the experiment, where the mean number of bites at the latter experimental days was significantly higher than the relative one at the early days (Figure 24a).

Variations on net biting were also present within the different periods of the day, for the three net conditions. Bites on the MN pen mainly occurred early in the morning. Sea bream

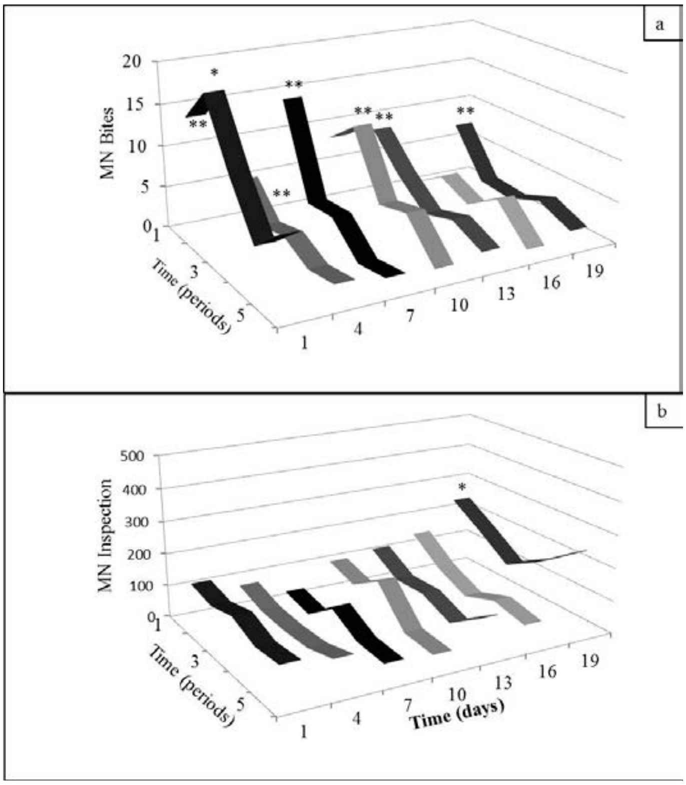

Figure 22: 3D analysis of fish activity on the MN group throughout days and time periods: a) Net biting (number of incidents), b) Net inspection (duration in frames). Statistically significant differences between days and time periods are presented by an asterix, relatively.

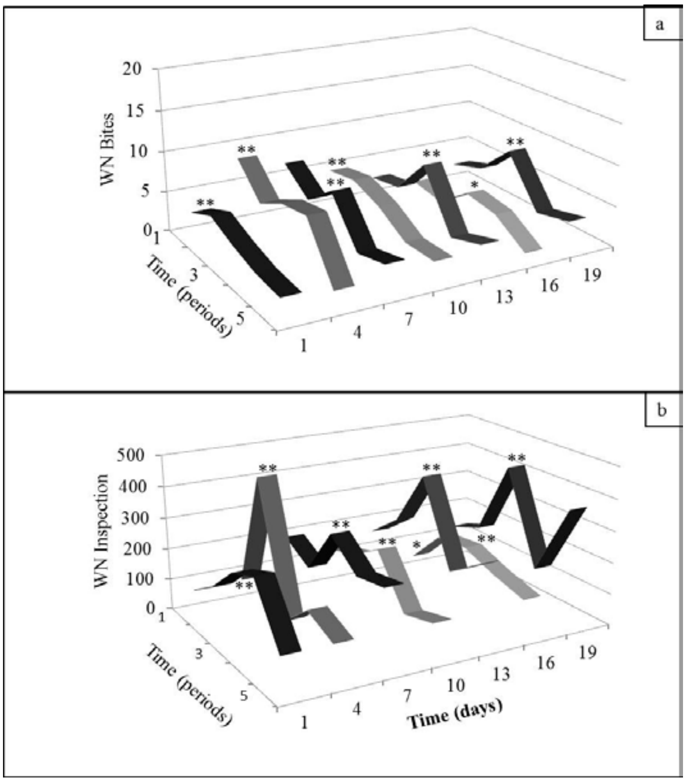

Figure 23: 3D analysis of fish activity on the WN group throughout days and time periods: a) Net biting (number of incidents), b) Net inspection (duration in frames). Statistically significant differences between days and time periods are presented by an asterix, relatively.

presented significantly less motivation to bite the net during the feeding time and until the end of the day (Figure 22a). In the case of the WN, number of bites was increased during the morning and the feeding time period, while it was significantly reduced after feeding and until the end of the day (Figure 23a). A different pattern was observed on the CN, where net biting was lower during the first hours of the day (Figure 24a). Lastly, fish activity later in the day was similar presenting a reduced bite activity in all net conditions.

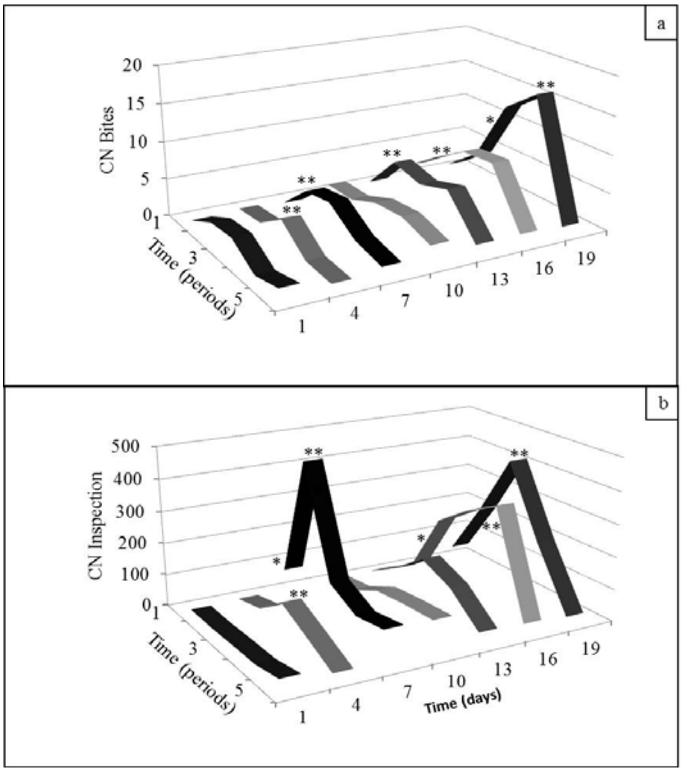

Figure 24: 3D analysis of fish activity on the CN group throughout days and time periods: a) Net biting (number of incidents), b) Net inspection (duration in frames). Statistically significant differences between days and time periods are presented by an asterix, relatively.

4.4.2. Net inspection

The in between treatment comparisons showed significant differences on the overall time spent for net inspection between the MN, WN and the CN group. The mean value / individual of net inspection were 2.14 ± 0.25 seconds in the WN, three times higher than the remaining net conditions during the experiment. No difference was observed on the CN (0.74 ± 0.19 s) and the MN (0.69 ± 0.12 s) fish groups.

Analysis within treatments showed that net inspection on the MN group (Figure 22b) presented higher ($P < 0.05$) values at the end of the experiment. Particularly, the percentage of time spent was approximately 50 % higher ($P < 0.05$) than the remaining analyzed days, where no differences were observed. In contrast, activity on the WN group (Figure 23b) had no difference throughout the analyzed days. Exceptionally on days 10 and 16, a decrease ($P > 0.05$) on fish activity was observed in comparison with the remaining days. In the case of CN group (Figure 24b), a significant increase on the time spent was clearly presented from early to latter ($P < 0.05$) experimental days. Exceptionally on day 7, fish activity was higher ($P < 0.05$) than day 1, 4 and 10, 13. Lastly, similarly to MN group, the relative activity at the later experimental days was approximately 50 % higher as compared to the early days of the experiment.

Data were additionally analyzed within 5 selected time periods. A similar slope was found in the WN and CN group, where net inspection was significantly higher at the feeding time in comparison with the first and last time periods (Figure 22b, Figure 23b). The MN presented a different behavioral pattern regarding the inspection of the net with no differences within the day (Figure 24b). Moreover, significant differences were observed between MN, WN and CN group for each one of the five time periods.

4.4.3. Net condition

At the end of the trials, all nets were further examined under stereoscope. The WN and the CN pens had no sign of micro-fouling detected on their surfaces. Additionally, the CN pen maintained the green-brown color until the end of the experiment without any visible sign of wear. The abundance of the MN filaments was reducing throughout the days and small areas; free of micro-fouling appeared on the net surface by the end of the experiment.

4.4.4. Growth performance

The growth performance of the three experimental groups is shown in Table 1. The growth rates presented no significant differences for all fish groups, according to the SGR index. Furthermore, the final Condition Factor (CFF) was higher ($P < 0.05$) than the initial (CFI), indicating the “good” condition status of fish in tanks. All fish groups gained weight (10.43 ± 0.74 g). Mortality or cannibalism incidents did not appear during the experiment.

4.5. Discussion

In spite of the recent advances in antifouling technology (Guardiola et al., 2012), biofouling, nowadays, remains a threat for a sustainable aquaculture worldwide. Fish rearing in floating net cages promotes the problem by providing favorable conditions for the colonization and growth of the fouling organisms (Hodson et al., 1997).

The present study demonstrates that marine micro-fouling has an influence on sea bream behavior, and motivates farmed species to further interact with the aquaculture net. The risk of net damage is thus potentially amplified due to the effect of macro-fouling that in turn is included in sea bream feeding habits (Pita et al., 2002). Particularly, micro-fouling filaments induced farmed sea bream to bite the net.

Furthermore, the color of the net also influences fish behavior under captivity, with the CN presenting the lowest net interaction. Experiments were performed at indoor aquaculture facilities where water conditions remained constant. This allowed the study of sea bream behavior under the effect of micro-fouling and the color of the net, avoiding the deleterious effects of biofouling. This would be limited in real aquaculture conditions since these effects would have interfered with the behavioral variations observed in the present study.

From the results obtained, fish activity in all groups was increasing during the day and by the feeding time, while was reducing until the end of the day. It seems that WN and CN groups have a positive linear relation between the two activities (net inspection and biting). Particularly, an elevated net inspection activity appears together with an elevated net biting, in both days and time periods. In contrast MN group showed a negative correlation between the two activities. Net inspection on the MN group remained very low because sea bream seemed to immediately locate and interact with the micro-filaments. This correlation was differentiated on the later experimental days, where the micro-fouling was minimal.

4.5.1. Net biting

This specific behavioral trait may have its origin to the species natural feeding. Sea bream appears to express this innate characteristic on the net, when in captivity (Glaropoulos et al., 2012; Papadakis et al., 2013; Papadakis et al., 2012). Specifically, sea bream was largely attracted to bite the fouling filaments on the MN group. The high number of bites that occurred on the present study clearly demonstrates that sea bream often bite the aquaculture net.

Intense bite activity has been previously related with the condition of the net. Sea bream individuals presented an elevated bite activity due to the presence of cut twines (Glaropoulos et al., 2012). The interest of fish for the cut twines in this study can be explained by their similarity with the micro-fouling filaments. This is consistent with the present study, where a similar behavior was found on the MN group during the first day. The significant reduction of these filaments at the end of the first day was strongly related with the number of bites along the experimental days.

The WN group presented no significant differences on net biting throughout the experimental period. The same white color aquaculture net has already been tested in similar behavioral studies under laboratory conditions; Sea bream expressed a constant bite activity towards the white net pen since the beginning of the trials (Papadakis et al., 2013). On the other hand, sea bream was less motivated to bite the green-brown color of the CN group. The white color net causes a higher contrast on fish view than the green-brown colored net due to their differences in light absorption and the scattering of the lower wavelengths of light from the sea water that acts as the background. Fish are able to distinguish colors while differences in colored nets have found to have an influence on fisheries catch (Balık & Çubuk, 2001). In this study the green color presented the higher number of catches probably driven by the less contrast on fish view. This might be the explanation of fish less motivation to bite the colored net pen in the present study. However, the elevating bite activity on the CN group at the later experimental days could be correlated with sea bream getting familiar with the net pen color and/or search for food, according to similar studies (Glaropoulos et al., 2012; Kristiansen & Ferno, 2007; Papadakis et al., 2013).

Significant variations were observed on net biting within the day. The intense bite activity on the MN group may be originated by sea bream hunger within the day (Montero et al., 2009). Particularly, fish were fed once per day, with 2% of their initial total weight, while they were left unfed 24 hours until the next feeding period. The aforementioned behavioral changes within the day have already been associated with an intense activity and prey search under limited-food conditions (Goldan et al., 2003). The micro-fouling filaments acted as additional stimuli that further motivated sea bream to bite the net. In contrast, net biting within the day, in the WN and the CN group, was affected by the color of the net. Sea bream is familiar with the green-brown color of its natural habitat (Tancioni et al., 2003) as compared to the "strange" white net. Therefore, sea bream was found to be motivated more by the white than the colored net. This comes in agreement with other studies that demonstrate that the natural mid-wavelength color environment (green) is the most favorable for the rearing process (Luchiari & Pirhonen, 2008). Generally, sea bream is a marine species that presents lower swimming activity and metabolic rate during the night (Angeles Esteban et al., 2003). It is likely that sea bream activity was reduced in all net conditions by the end of the day late in the day (5th time period).

4.5.2. Net inspection

Confined fish in tanks i.e. Atlantic cod (Moe et al., 2007) and sea bream (Papadakis et al., 2013) have been largely observed to approach (<2 cm) the net pen, an activity that is commonly known as net inspection. This behavior increases the risk of net damage, considering that net biting could follow net inspection. In the present study it is shown that the color of the net has an influence on fish inspection.

Related studies showed that sea bream inspection is associated with the condition of the net. Particularly, the existing cut twines further induces this activity (Glaropoulos et al., 2012; Papadakis et al., 2013). As previously mentioned, the micro-fouling filaments acted as a stimulus resulting to fish attraction as compared with net cut twines. Fish inspection in the MN group was observed from the beginning of the experiment. The elevated time spent for net inspection by the end of the experiment may be originated by the lack of such filaments. Sea bream seemed to be mainly interested in biting those filaments and so, increased net inspection may indicate search for any remains.

Net inspection followed a similar pattern in WN and CN group as compared to the previously described bite activity. Particularly, sea bream was less motivated to approach the green-brown net. The WN group showed an increased inspection throughout the day in comparison with the CN group. Moreover, this activity was increasing until the feeding time, indicating that the amount of food provided to fish is also related with an intensified activity, originated by hunger.

4.6. Conclusions

The present research has involved three net conditions commonly found in aquaculture in order to investigate fish attraction towards the net pen. In contrast to other related study (Guenther et al., 2009) that evaluated the effect of several net colors on the settlement of fouling species, this is the first study that demonstrates that sea bream is attracted by the micro-fouling filaments and not so much by the color of the net tested. As previously mentioned, the overall net biting was no significant different between treatments. Nevertheless, the analysis throughout days and time periods showed significant differences between treatments that lead to better understand the effect of different net condition on fish interaction. It is clearly demonstrated that the presence of micro-fouling highly induces sea bream motivation to interact with the net pen. Additionally, the white color of the net causes the higher net attraction due to the intense contrast on the confined fish view. In contrast, the CN group presented the lowest overall attraction on the net. Lastly, it can be concluded that sea bream attraction on the micro-fouling net pen has its origin to the existing filaments and not the color of the net.

The use of a green-brown net pen is widely recommended in cage facilities, since farmed species are significantly less motivated to interact with such colored net. Different colors or net materials should be further evaluated whether they provide unfavorable conditions for sea bream bite activity.

5. Escape-related behaviour and light preference of European sea bass

5.1. Abstract

The escape behavior of European sea bass (*Dicentrarchus labrax*) under different light levels was evaluated in laboratory conditions. Fish were confined in tanks that were previously split into two equal compartments by a removable net pen. A tear on the net allowed the fish to move between the two compartments of the tank. A black net frame was placed upon on half of the tanks to differentiate them in light conditions. Three combinations were tested in triplicates: light - shade, shade - light and a control condition light - light. Sea bass located the net tear and crossings occurred in all tanks, regardless of light condition. When differences in light level were present, sea bass showed a clear preference for the illuminated areas of the tank, with fish aggregation in the light areas of the tank observed throughout the entire experimental period. The fish did not seem to be willing to return to the shadowed areas, even if they have to starve, raising crucial concerns towards a sustainable aquaculture. The results open up for new discussion of using artificial lights to prevent large-scale escapes in commercial-scale aquaculture.

5.2. Introduction

Escape and pre-escape behavior has already been evaluated in many farmed species (Glaropoulos et al., 2012; Jensen et al., 2010; Moe et al., 2007; Papadakis et al., 2013; Papadakis et al., 2012) and also under several biotic and abiotic factors. In commercial-scale aquaculture, escapes have been mostly reported after severe environmental conditions and damages on cage structure (Jensen et al., 2010). In addition, only scarce information exists on the influence of light on the escape behavior in aquaculture.

Light is an important abiotic factor that has been associated with fish biology and behavior. Most fish are based on vision system to perform activities such as foraging and breeding. Thus, artificial light may also have an influence on fish behavior. The use of artificial light has been tested in fisheries studies (Marchesan et al., 2005) and also in cage production (Oppedal et al., 2001; Vera & Migaud, 2009) so as to control and manipulate fish essential activities, like swimming speed (Juell & Fosseidengen, 2004) and schooling behavior (Castro & Caballero, 2004; Noble et al., 2005). In addition, selective lights have also been applied on fisheries research to obtain more efficient catches. Particularly, sea bream and grey mullet are strongly attracted by high illumination levels whereas sea bass presented a moderate attraction either to light or short wavelength (Marchesan et al., 2005). In view of the importance of light as environmental driver in commercial cages, almost no study has been carried out to describe the escape behavior of farmed species.

European sea bass (*D. labrax*) is of high commercial interest in European aquaculture (Villamizar et al., 2011). The pre-escape and escape behavior of sea bass has already been evaluated under experimental conditions, concluding that sea bass is neither capable of directly damage nor interact with the net pen but rapidly escapes at the presence of a net tear. It has been found that sea bass escape behavior is related to the visual conditions around the cage environment (Papadakis et al., 2013) and particularly, it can be significantly delayed when a visible obstacle exists at the point of escape. Therefore, research should be focused on the environmental drivers that may influence sea bass to cross a hole on the net.

The objective of the present work is to examine the fish preference on light by studying their escape behavior, using sea bass juveniles as a test sample. Whether variations on light level affect sea bass behavior it is crucial for fish management and prevention of large-scale incidents. Our study was performed in tanks under fully controlled conditions, where fish are able to express preference between lightened and shadowed areas. The present study simulates commercial scale aquaculture, where variations in light intensity around the cages might affect fish motivation to escape.

5.3. Materials and methods

5.3.1. Experimental fish

A total number of 135 sea bass individuals (weight: 26.73 ± 1.05 g and length: 13.34 ± 0.25 cm) were provided by the Institute of Aquaculture of the Hellenic Centre for Marine Research (HCMR). Fish were randomly selected from an initial large population that had been reared with the mesocosm's methodology (Divanach & Kentouri, 2000) in 40 m^3 tanks and following in 10 m^3 pre-growing tanks. Sea bass individuals were then transferred to the University of Crete, and confined into experimental tanks.

5.3.2. Tank preparation

Fish groups (15 individuals / tank) were kept in groups to acclimate for one week prior to the beginning of the experiment. Following acclimatization, each tank was split into two equivalent areas (50 % of the volume) by a removable net pen (white polypropylene net, 17 mm hexagonal mesh opening) fixed in a plastic frame (31 x 28 cm) that was fitted tightly to the bottom and the two sides of the tank. A tear (5.1 cm height) located centrally on the net pen, allowed fish to cross between the compartments of the tank. Fish were initially confined to the left area of each tank, referred to as the "holding" area, while the area on the right side was referred to as the "escape" area.

Prior to the beginning of the experiment, a plastic, black color frame (length 55 cm, width 34 cm) was placed upon the tanks to differentiate them in three light conditions; 1) Illuminated Holding Area (IHA) - Shadowed Escape Area (SEA), 2) Shadowed Holding Area (SHA) - Illuminated Escape Area (IEA) and 3) Illuminated Holding Area (IHA) - Illuminated Escape Area (IEA) as control condition. These conditions were tested for a total time period of 13 experimental days. Light intensity in every tank compartment was measured with a Light Meter (EXTECH INSTRUMENTs, L825251), and was 330 ± 7 lux in the illuminated area and 8 ± 2 lux in the shadowed area. Both before and during the experiment, fish were fed ad libitum once every day (at 14.00 h) by hand with commercial sea bass pellets (Biomar INICIO Plus No 1,9: 58 % crude protein; 18 % crude lipids). Food was always provided at the left side of the "holding" area.

5.3.3. Experimental design

Fish groups (15 individuals / tank) were left in tanks to acclimate for one week prior to the beginning of the experiment. Following acclimatization, each tank was split into two equivalent areas (50 % of the volume) by a removable net pen (white polypropylene net, 17 mm hexagonal mesh opening) fixed in a plastic frame (31 x 28 cm) that was fitted tightly to the bottom and the two sides of the tank. A tear (5.1 cm height) located centrally on the net pen, allowed fish to cross between the compartments of the tank. Fish were initially confined to the left area of each tank, referred to as the "holding" area, while the area on the right side was referred to as the "escape" area.

Prior to the beginning of the experiment, a plastic, black color frame (length 55 cm, width 34 cm) was placed upon the tanks to differentiate them in light intensity.

Three different conditions; Illuminated Holding Area (IHA) - Shadowed Escape Area (SEA), Shadowed Holding Area (SHA) - Illuminated Escape Area (IEA) and a control condition that referred to an Illuminated Holding Area (IHA) - Illuminated Escape Area (IEA) were tested for a total time period of 13 experimental days. Light intensity in every tank compartment was measured with a Light Meter (EXTECH INSTRUMENTs, L825251), and was 330 ± 7 lux in the illuminated area and 8 ± 2 lux in the shadowed area. Both before and during the experiment, fish were fed *ad libitum* once every day (at 14.00 h) by hand with commercial sea bass pellets (Biomar INICIO Plus No 1,9: 58 % crude protein; 18 % crude lipids). Food was always provided at the left side of the "holding" area.

5.3.4. Data acquisition

Fish activity was monitored by an upgraded version of the computer vision system developed for this purpose (Papadakis et al., 2012). The system consisted of nine color digital CCD cameras (Fire-i, Unibrain) placed in front of the tanks. The cameras' acquisition rate was set to 9 frames per second and the cameras were recording continuously from 8.00 until 20.00 o'clock daily for the entire duration of the experiment. Influence of human presence on fish behavior was limited to the feeding time, since observations of fish activity in all tanks were achieved through a remote, real-time web-server (LabView, Web Publishing Tool). Night observation was not important since the parameter under consideration (light condition) was not valid during the night.

5.3.5. Data analysis and statistics

All acquired video data were analyzed with the use of custom-made software (Papadakis et al., 2012), written in LabView (National Instruments). All crossing attempts through the net tear to the other side of the tank were recorded – both from the holding to the escape area and reversely. The statistical analysis of the data was performed accordingly to related studies (Glaropoulos et al., 2012; Papadakis et al., 2013; Papadakis et al., 2012) based on similar experimental setup, using ANOVA (SIGMASTAT statistical package; Systat Software, San Jose, Calif.).

The first experimental day was fully analyzed and the exact number and time of all escape and return events were recorded. This analysis was restricted to the first day, since no fish remained in the SHA by the end of the day. Learning was assumed to be neglible during this time period, since fish had been previously acclimatized under no difference in light intensity. Fish activity was presented in the format of mean value ± SE for each triplicate (three conditions). Statistical analysis consisted of two-way ANOVA, where the number of crossings (escape, return) was set as the dependent variable while the light condition and tanks were the fixed variables. No differences were observed between replicates ($p > 0.05$).

After the first day, sea bass were not only confined in the initial holding area of each tank, which did not allow the study of fish reaction to light intensity. Therefore, for the remainder of the experimental days (day 2 to 13), a different statistical approach was followed. Five time periods of 15 minutes each within the day (starting at times: 9:00 early morning - 11:00 morning - 13:30 before feeding time - 15:30 after feeding time - 18:00 late afternoon) were selected so as to analyze crossing activity under different light conditions and thus species preference to light intensity.

Statistical analysis consisted of a three-way ANOVA. The dependent variable was the number of crossings that occurred, while the fixed variables were the experimental days, the tanks as well as the five time periods within the day. Differences between triplicates and the five time periods among the experimental days were tested and no significant variation was observed between the three light conditions.

The development over time of the crossing activity among the experimental days was examined by calculating the mean crossing value ± SE of each light condition including the five time periods of each day. Variations between the five time periods were also investigated, calculating the mean value ± SE of crossing incidents, adding the activity from tanks of each light condition and all experimental days.

Normality tests (Shapiro-Wilk test) were performed so as to control the distribution of the data. When the data did not follow a normal distribution, non-parametric control Kruskal–Wallis and Mann–Whitney tests were applied. All Pairwise Multiple Comparison Procedures were tested using Tukey Test. Fish were not individually tagged or recognizable. Data were treated group-wise (n=3). The level of significance was set at 5 % ($p > 0.05$). For all mean values, standard error (SE) was calculated.

5.4. Results

5.4.1. First experimental day

Early in the first day, fish had to face the new light conditions in tanks. During the first hours, fish remained motionless and mainly aggregated close to the bottom of the holding area. The activity increased within the day and sea bass started pushing the net pen. The first escape occurred in the control condition, while more incidents followed the first event in the other tanks and light conditions. Most of the escapes occurred in series of two (2) or even seven (7) individuals. No incident of mortality or cannibalism was observed during the experiment. The different light conditions between the two compartments of the related tanks had an influence on the exact time of the first escape and return. In fact, fish needed more time (5 minutes) to locate and cross the net tear, from the IHA or the SHA group.

Sea bass in the IHA group showed a strong preference ($q > 14.7, p < 0.05$) for light during the day, and half of the group (50 %) still remained in the IHA of the tank by the end of the day (Fig. 1). The first escape was recorded in a series of two individuals, 4 minutes after the beginning of the experiment.

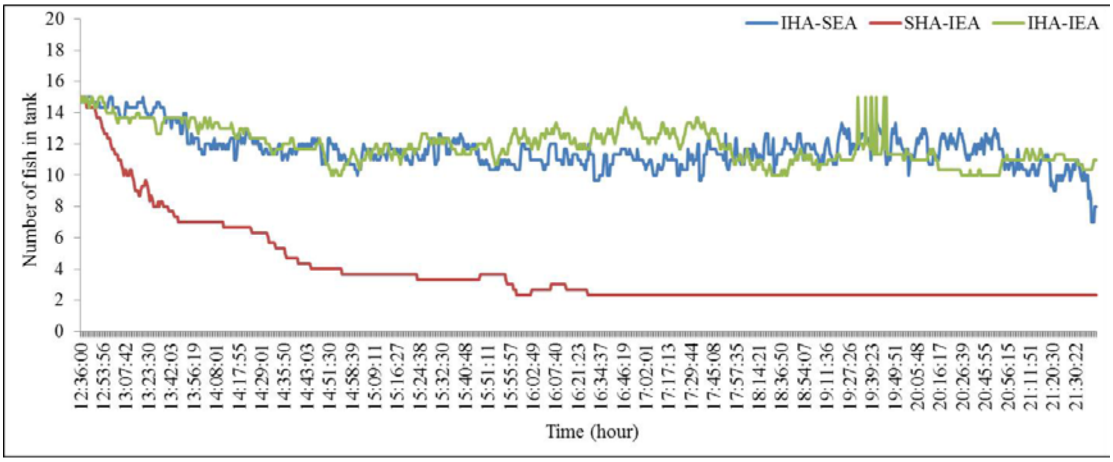

Figure 25: Variations of fish population confined into the holding area of the tank until the end of the first experimental day. Measurements are formatted as mean value ± SE, for the three light conditions.

A significant number of escapes to the SEA took then placed within the first day but they were all followed by an equal number of returns to the IHA of the tank. The largest crossing event observed in this group was in a series of seven individuals. Fish were mainly observed close to the net pen, either on the illuminated area or in the shade, resulting into an uneven fish distribution several times within the day.

In contrast, fish initially confined in shade (SHA) started crossing the net tear and moved to the escape area (IEA), with more light. The first escape occurred at about the same time (5 minutes) as in the previous light condition. By the end of the first day, 80 % of fish population had moved to the higher illumination area, while no return incidents were observed (Figure 25).

In the control condition (no difference in light level), only a few escapes were recorded during the first hours of the experiment. Fish were mainly distributed in the bottom of the tanks, showing a strong schooling behavior.

5.4.2. Experimental days 2-13

Illuminated Holding Area – Shadowed Escape Area

Fish were mainly distributed (80 % of the population) in the illuminated area of the tanks. Similarly to the first day, crossing activity continued during the experiment, with the same number of escape and return incidents. However, the number of crossings was higher ($F > 2.23$, $p > 0.05$) in the second and third day (Fig. 2a). The time interval between an escape and a return incident was calculated to be approximately 8 seconds on average in a total of 350 measurements. Additionally, the number of crossings into the shadowed area of the tank was reduced along and until the end of the experiment.

Shadowed Holding Area – Illuminated Escape Area

During the first experimental day, almost all fish had escaped to the illuminated area of the tank. Fish were limited motivated (5-10 %) to cross the net tear and return to the holding area of the tank. Crossing activity was almost no presented until the fifth experimental day. Sea bass first attempts to return to the initial holding area started on the sixth day (Fig. 2b). Random tests on video recording at the feeding time revealed remains of uneaten pellets at the bottom of the tank that clearly confirmed the unwillingness to return to the shaded area.

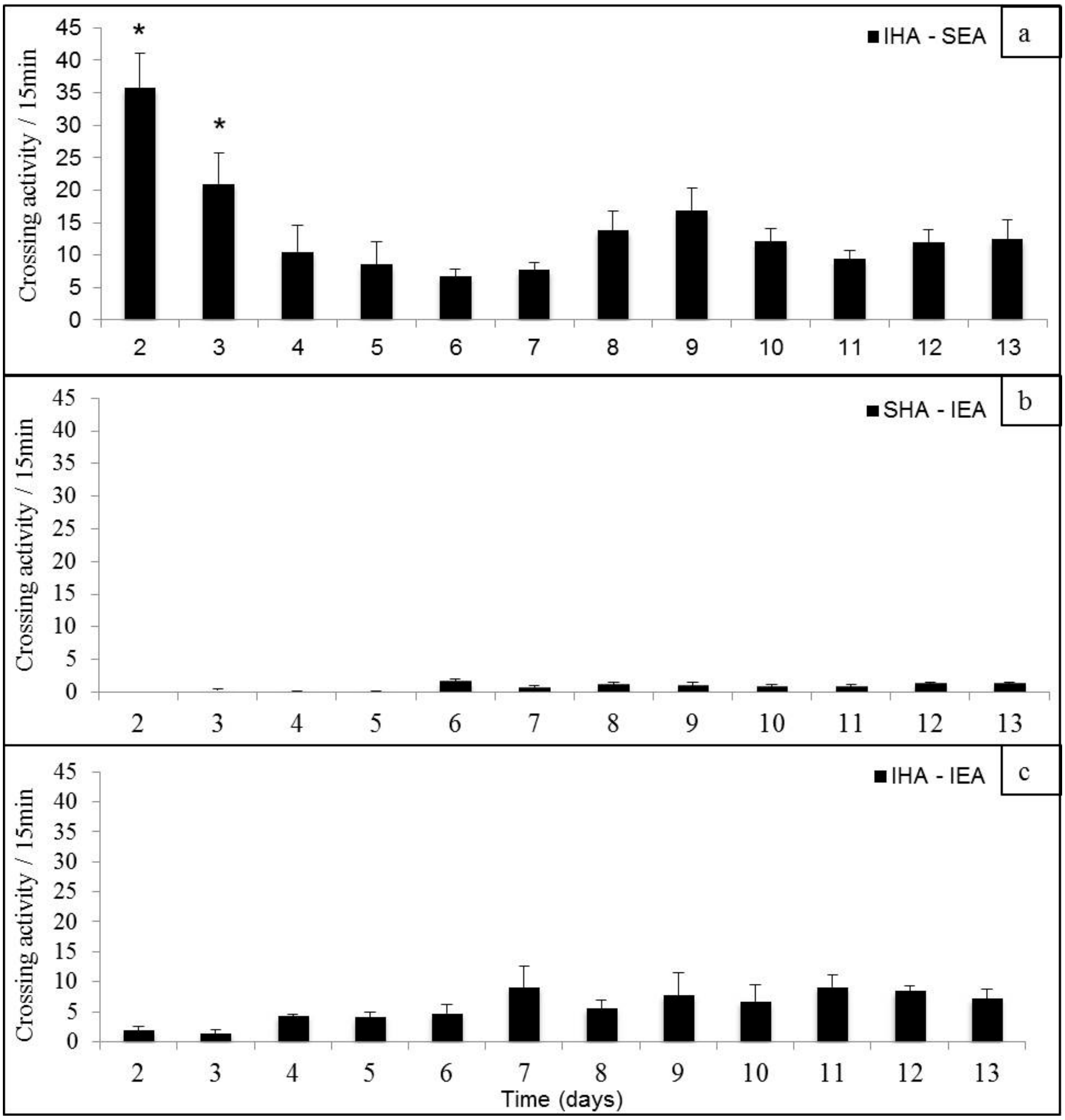

Figure 26: Number of crossings that occurred from day 2 to day 13 of the experiment, in the three light conditions. Data are presented as mean value ± SE. Statistically significant differences are indicated with an asterisk.

Illuminated Holding Area – Illuminated Escape Area (control condition)

The schooling behavior that initially characterized fish activity in this group continued. Crossing activity was quite low on the first days, with fish generally observed at the bottom of the holding area. During the following experimental days, the fish gradually increased their activity (Figure 26c). In general, at the end of the experiment fish distribution was even (55-45 %) between the two areas of the tank.

5.4.3. Crossing activity within the day

Differences on crossing activity between the light conditions and within the day (five time periods) were observed. Fish activity between triplicates of each light condition was similar within the five time periods of the day. A further analysis showed that crossing activity was correlated with time of day. Fish was more ($q > 0.09$, $p > 0.05$) willing to cross the net tear after the feeding time in all experimental days, regardless the light conditions that existed in tanks (Figure 27). Additional analysis showed that the control condition presented higher crossing activity during this specific time period than the other tank groups (Figure 27a, b, c).

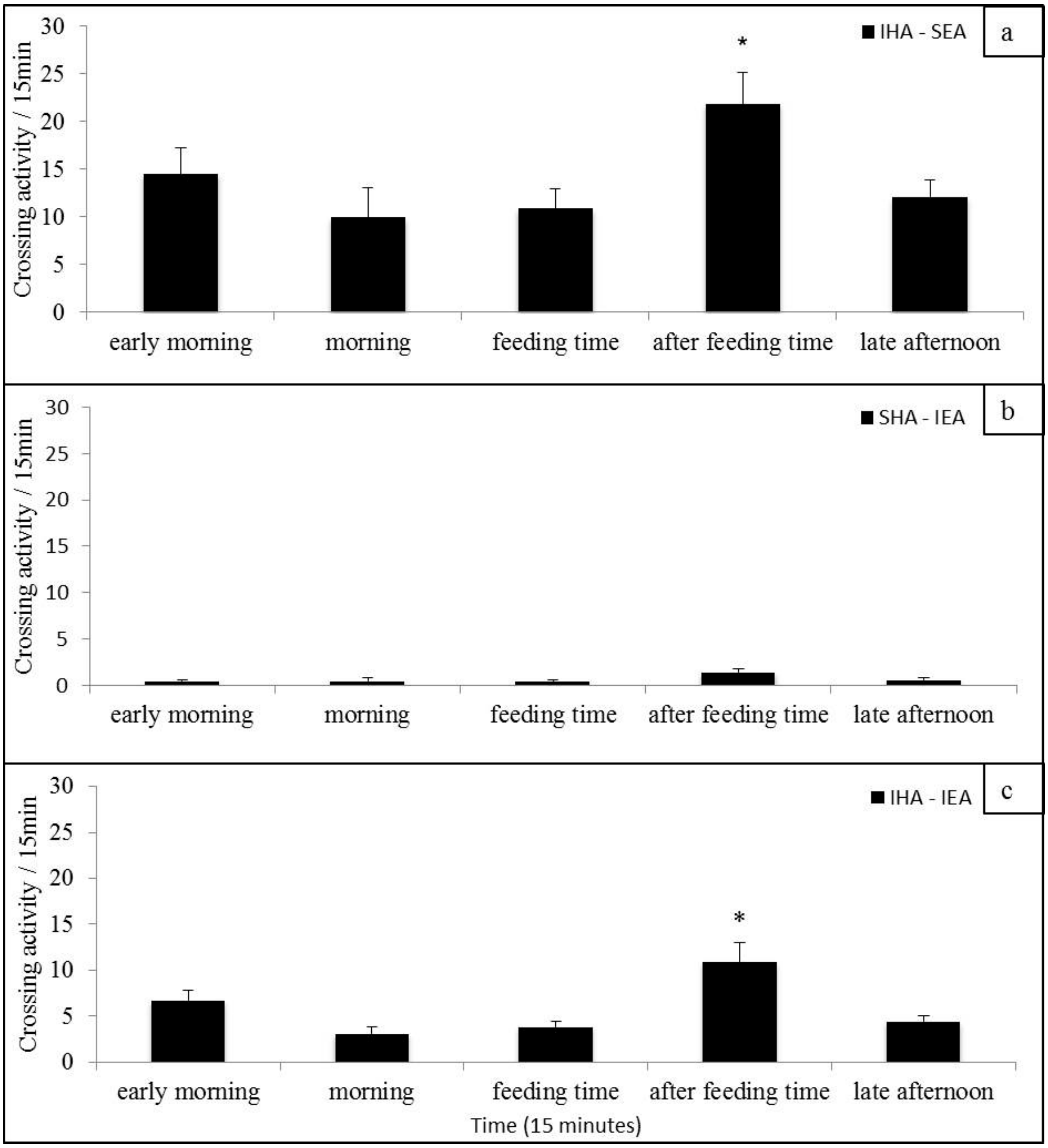

Figure 27: Time-dependent crossing activity of sea bass individuals in five time periods of a day. Data are presented as mean value ± SE. Statistically significant differences are indicated with an asterisk.

5.5. Discussion

Sea bass is an important farmed species (80 K tons in 2005) in the Mediterranean aquaculture (Benhaim et al., 2013), and has been reported to escape from sea cage installations (Triantafyllidis, 2007). The factors of influencing escape (Naylor, 2006) as well as the major impacts of the problem have been extensively documented during the last years for a variety of farmed species (preventescape.eu). Our results clearly indicate that sea bass is able to locate any tear on the net pen and escape; regardless of the differences in light condition between the holding and the escape areas. However, the behavioral observations revealed that different light level in tanks has an influence on sea bass escape behavior.

European sea bass is a finfish characterized by an elongate body size (Volcaert et al., 2008) and a remarkable swimming activity (Pickett & Pawson, 1994). These characteristics along with the small volume of the tank may have contributed to the location of the tear and the ability of the species to cross the net tear and escape.

The results obtained from the first experimental day clearly demonstrate that *D. labrax* juveniles showed a preference for the illuminated areas, either remaining or moving towards there, even if they had to starve. This attraction may have to do with that fish were previously acclimatized for a period of seven days, in illuminated light conditions. The sudden creation of shade on the SHA tank group could act as a stimulus to increase their escape activity. Sea bass individuals might become stressed by the sudden light change and crossed the net tear to the other side of the tank, with more light. This reaction was stronger than the reaction to food, since a significantly low number of returns was observed even at the feeding time, throughout the experiment. The number of uneaten pellets that were found at the bottom of this tank group (SHA) confirmed the unwillingness to return. Food deprivation of 5 experimental days may explain why a few sea bass returned to the dark area and detect the food, even under low illumination level. In the case of a longer experimental period, return activity might have increased to reach the activity of the IHA-SEA group. Related studies with Atlantic halibut (Yacoob & Brownman 2007) and sea bream juveniles (Glaropoulos et al., 2012) also revealed intense escape activity, probably driven by search of food. However, in this case, over 15 days of food deprivation was required for high number of sea bream crossings.

The significant number of escape and return incidents observed in the IHA-SEA group, could be explained by exploitation of the tank and search for food. European sea bass as well as other marine species (mackerels, carangids and tunas) tend to move in and out of the illuminated field driven by feeding motivation. In our study, sea bass was mainly located in the illuminated areas of the tank, close to the net pen, on the border between light and shade. This is consistent with (Marchesan et al., 2005) observations, while in our study, the net pen that split tanks in half resulted to a more complicated behavior. The fish escaped to the shadowed area individually, spent some time on shade (8 seconds on average), and returned to the illuminated area of the tank. Schooling behavior, which is a characteristic behavioral trait of sea bass, along with their preference to light areas, could explain the short presence in shade, since most of the population was still located in the illuminated area.

When there was no difference in light condition in the tanks (control), sea bass showed an intense schooling behavior instead of crossing the net tear. Schooling behavior is quite common for this species, when confined into small tanks (Malavasi et al., 2004). Crossings gradually increased over the experiment probably due to exploitation of the tank. The highest crossing activity was observed after feeding time indicating that the fish returned to the holding area to eat and then again distributed themselves evenly in the whole tank volume.

In conclusion, it seems that swimming and crossing behavior of sea bass is strongly affected by the light conditions. A strong light preference in tanks was observed in the present study, which suggests that a similar attraction is present in the cage environment. Further, sea bass preference to the lighten area, even if they have to starve clearly indicates that the parameter of light should be greatly considered in aquaculture industry, since it could significantly influence fish growth and welfare.

An artificial light after storm or severe environmental conditions might keep sea bass aggregated close to the light source and away of possible holes on the net pen. Implications of artificial light in commercial aquaculture could minimize the risk of large-scale escape. A possible problem is that variations in light level within the cage may cause fish to exploit only a small part of the cage volume, with negative effects on feeding, growth and welfare. The appropriate management of the cages' location and orientation is highly recommended so as to provide the optimal light condition to the entire area of the farm facilities.

6. Do the visual conditions at the point of escape affect European sea bass escape behavior

6.1. Abstract

European sea bass (*Dicentrarchus labrax*), an important species for the Mediterranean aquaculture industry, has been reported to escape from sea cage installations. Fish escapes are caused mainly by operational and technical failures that eventually result into a creation of a tear. Escapees may interact with wild stocks through interbreeding, transfer of pathogens and competition for food. The aim of this study was to examine at which extent the presence of a visible obstacle close to a tear on the net have an influence on sea bass propensity to escape. Fish were initially confined into small sea cages, with a tear at one side. The escape behavior was tested under experimental conditions. It is clearly demonstrated that sea bass was able to locate a tear on the net pen, immediately after its appearance. Crossings occurred in all cages, in singles or in a series of up to seven individuals. The presence of an obstacle close to the net tear altered the escape behavior of *D. labrax* resulting in a delay that eventually reduced the escape rate. Concluding, it is highly recommended that sea bass cages should be kept internally the culture array. Furthermore, the placement of artificial obstacles close to the sea cages could be an efficient practice that mitigates the escape risk after severe environmental conditions.

6.2. Introduction

Escapes of fish from sea cage installations raise a number of remarkable concerns on the marine environment (Naylor et al., 2005; Triantafyllidis, 2007). Fish that escape from farms may interact with wild conspecifics during spawning (Uglem et al., 2008) and interbreeding could threat the genetic integrity of wild populations (Jensen et al., 2010). Escapees may also compete for food with the wild. Another potential impact is the transfer of diseases and pathogens; for example, farmed salmon have been identified as reservoirs of sea lice in Norwegian coastal waters (Heuch & Mo, 2001). Thus, there is a number of ways in which escapees have an impact on the natural environment (McGinnity et al., 2003).

Along with the environmental impacts, economic issues are also detrimental to both aquaculturists and companies. Company's reputation as well as conflict with environmental groups is considered as the most important consequences (Jensen et al., 2010). Additionally, replacement of the damaged equipment and also recapture of the escapees increase the overall cost for the aquaculture industry (Naylor, 2006).

Such escapes have been reported for almost all species that are reared in the European aquaculture (*Sparus aurata, Dicentrarchus labrax, S. salar, G. morhua*) and occur at all the stages of the rearing process (Haffray et al., 2007), like induced breeding, larval stage and grow out (Jensen et al., 2010). This pan-European problem that also exists in many other countries still threatens the sustainability of the aquaculture industry.

The main cause for fish escapes is a combination of structural failures of equipment under severe environmental conditions. Several numbers of salmon's escape have been reported after intense coastal storms in Norway (Norwegian Fisheries Directorate, 2007). Fish predators attacking the cages may also result into creation of holes on the cage net (Dempster et al., 2002; Jensen et al., 2010). Recently, a significant number of studies have been focussed on the pre-escape behavior (fish inspection and biting the net pen) of the farmed species, since it may lead into damages on the net pen and creation of holes (Moe et al., 2007). Atlantic cod (Hansen et al., 2008) and gilthead sea bream (Glaropoulos et al., 2012; Papadakis et al., 2012) are still the main farmed species in the European aquaculture, which regularly present the above specific behavior.

European sea bass is also mainly farmed in the Mediterranean Sea (Villamizar et al., 2011), and a significant number of escapes have been reported from sea cage facilities (Arechavala-Lopez et al., 2011) immediately, when a tear appeared on the net pen. No interaction with the aquaculture net pen has been documented, and sea bass does thus not seem to be able to cause damages, create holes and escape. Based on the above statements, it is important to define factors that lead fish to locate a tear on the net and escape.

The aim of this study was to evaluate the escape behavior of European sea bass as related to the visual conditions at the point of escape. Particularly, it was examined whether an obstacle in front of the tear affects the propensity of fish to escape. Also, we tried to evaluate at which extent the type of the obstacle influenced this specific behavior.

6.2. Materials and methods

6.2.1. Experimental fish

The study was carried out at the Institute of Aquaculture of the Hellenic Centre for Marine Research (HCMR). Two successive experiments were performed, for 17 days respectively. Larval rearing process until fish metamorphosis was performed with the technology of mesocosm (Divanach & Kentouri, 2000) in 40 m^3 tanks. After that, fish were transferred to 10 m^3 pre-growing tanks, where they were kept for 150 days, under the same rearing conditions. A total number of 420 juvenile sea bass were used in the study. Fish were randomly selected from the initial large group and sequentially allocated into experimental groups (35 individuals / group). All individuals had approximately the same weight (110 ± 22 g) and length (24.6 ± 1.2 cm).

6.2.2. Sea cages preparation

The experiments were performed into six handmade sea cages (length 60 cm, depth 80 cm and width 60 cm) that were placed in a 17 m3 semicircle tank. Common white-color aquaculture net was used to construct sea cage's net. A tear was created at one side of the net, while was large enough (5 cm) allowing fish to escape. Natural sea water renewal was achieved under a constant flow (10 %/h). The temperature was 20oC, the salinity was 38 ‰ and the dissolved oxygen was above 95 %, throughout the whole experimental period. Natural photoperiod (daylight: from 06:00 a.m. to 08:00 p.m.) existed during the experiment.

6.2.3. Experimental design

The cages were placed in an array formation of two by three, inside the tank. The first line of three cages (1st group) was placed opposite to the front wall of the tank (distance <60 cm) and the second line (2nd group) right behind them (distance <30 cm). According to the above design, there were two cage groups in the tank: the "internal group", where the tear faced the wall of the tank (<60 cm) and the "external group", where the tear faced the open view area of the tank (3.5 m from the distant wall of the tank). The distance from the bottom of the tank was 1.8 m and it was equal for the two cage groups.

Thirty-five (35) fish individuals were initially confined in each one of the six sea cages. Food supplied once per day, equally for all six cages (2 % of the initial total body weight of fish population per cage). Food was also provided manually and in small amounts, inside the cage environment, to give the fish enough time to consume the sinking pellets.

Two subpopulations from the initial one were used in the following two experiments. In the 1st experiment, the tear at the internal cages was facing the front black wall of the tank, while the tear at the external cages was facing the open view volume of the tank. In the 2nd experiment, a net curtain (length: 3 m, width: 2.2 m) was placed in front of the tear of the external cages (<60 cm) and until 30 cm from the bottom of the tank, acting as an obstacle such as the wall of the tank at the internal group (Figure 28).

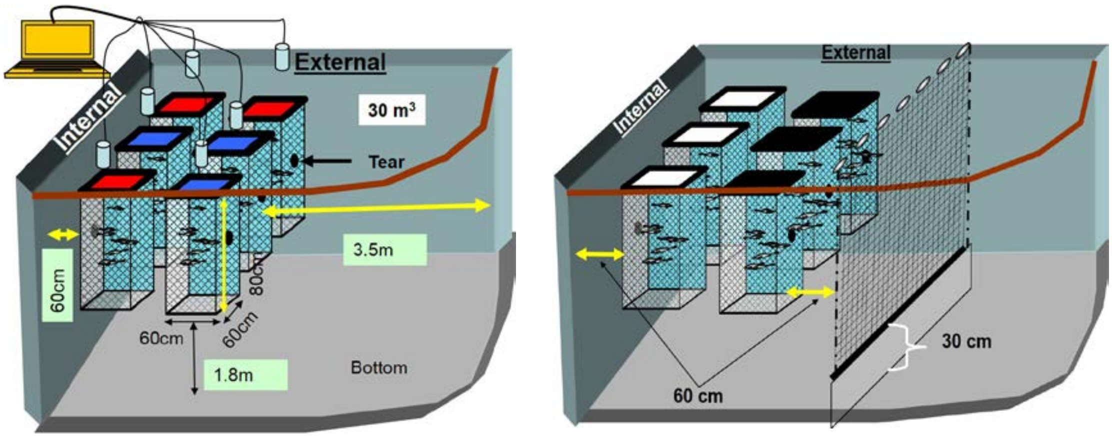

Figure 28: Handmade sea cages submerged in a 30 m^3 tank during the 1st experiment A (left) and the experiment B (right).

6.2.4. Monitoring fish activity into the cages

Six external cameras that were installed above the cages, each one recording a single cage, allowed observations of fish activity into the sea cages. The cameras were all connected to a computer, located outside of the experimental area, eliminating the influence of human presence on fish behavior. Acquisition was performed through a multi-camera frame grabber (GV-1120, Geovision), able to simultaneously record video data from all cameras. The requested frame rate was set to 30 frames per second. Recording of videos was pre-set to start daily from 07:00 a.m. until 07:00 p.m., where natural light variations were acceptable. Then, the video stopped and started again the next day. Data of each day were transferred to the computer and stored as avi file.

6.2.5. Data extraction and analysis

All acquired video data were visually analyzed, with a use of Windows Media Player. A whole day (07:00 a.m. to 19:00 p.m.) analysis was performed so as to analyze sea bass escape activity for all sea cages and for all experimental days.

The total number of escapes was measured in every sea cage, referring to the number of incidents that one fish cross the tear to the outer space of the cage. The exact time of every single incident was also recorded so as to provide detail information of the escape behavior of *D. labrax* individuals. Lastly, the remaining fish population into the cages was counted at the end of each experimental day so as to provide the overall remained fish population.

Further analysis was also performed in order to calculate the % escape rate (number of escaped fish per hour x 100) of sea bass individuals. This rate was only calculated for the first experimental day, since in the first experiment, all external cages were emptied by the end of this day.

Additional analysis was performed in order to compare the observed variations on the escape activity of *D. labrax* due to the presence/absence of the net obstacle. The remaining fish population in both the external and internal cages was measured by the end of each day. Then, the normalized remaining number of fish (external/internal), referred as NRNF, was calculated for each experiment, dividing the external population by the internal one, for each experiment.

6.2.6. Statistical analysis

The fish were not individually tagged or recognizable (tracked). Statistical analysis was performed using two-way ANOVA (SIGMASTAT statistical package; Systat Software, San Jose, Calif.).

The experimental day and also the position of the cage were kept as the fixed variables, while the number of escapes was set as the dependent variable.

When the data were following a normal distribution, the differences between the groups were detected using the Student–Newman–Keuls test; when the data did not follow a normal distribution, nonparametric control Kruskal–Wallis and Mann–Whitney tests were applied. Data were treated group wise ($n = 3$). The level of significance was set at 5 % ($p<0.05$). In all the mean values, standard error was calculated.

6.3. Results

No incident of mortality or cannibalism was observed during the experiments. Sea bass individuals appeared to be motionless at the time that they were confined into the sea cages. After a short acclimatization period (<1 h) fish increased their activity and started pushing on the net pen but not actually biting it. In a matter of time, fish located the tear in the net pen and escaped. A high number of escapes were measured throughout the experiments. Many escape incidents were observed to be in series as shown in the video data. Differences on the number of escapes that occurred were associated with the visual conditions at the point of escape. There were no differences found (P > 0.05) between the replicates in any of the experiments.

6.3.1. Experiment A - Solid visual obstacle at the point of escape

The presence of the wall of the tank (solid obstacle) resulted to a lower (p<0.05) escape activity between the two cage groups. At the external cages, approximately 80 % of fish population escaped on the first day. This number increased to approximately 90 % by the end of the second day. By the end of day 5 and until the end of the experiment, no fish was observed inside any of the three external cages. In contrast at the internal cages, only 6 % of the initial fish population had escaped at the first day. Furthermore, approximately half (50 %) of their initial population still remained into the cages by the end of the experiment (Figure 29).

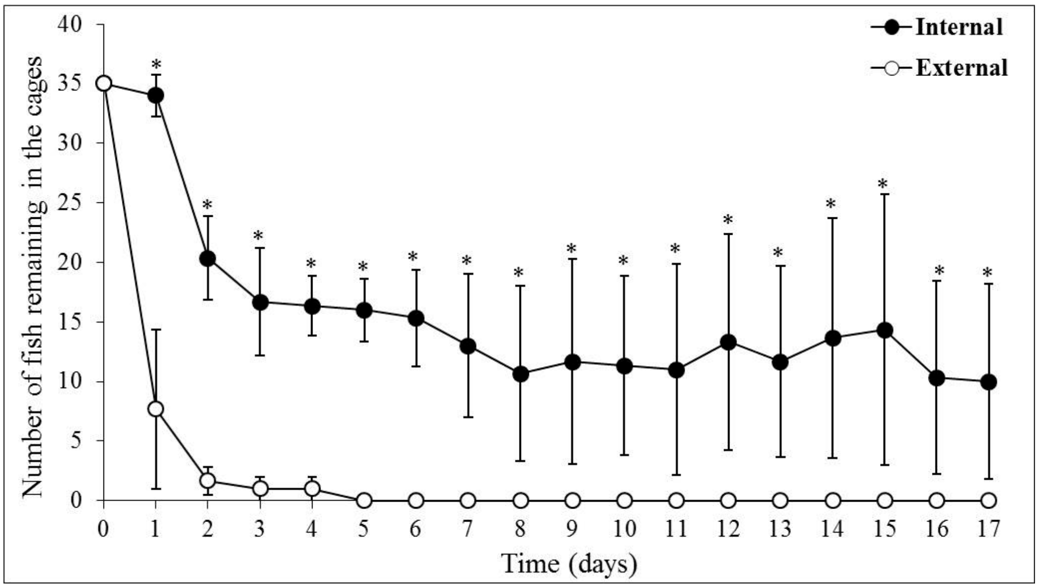

Figure 29: Number of fish (mean ± SE) remained at both internal and external cages by the end of each experimental day (17days) in Experiment A. Statistically significant differences between the two-cage groups are indicated with an asterisk.

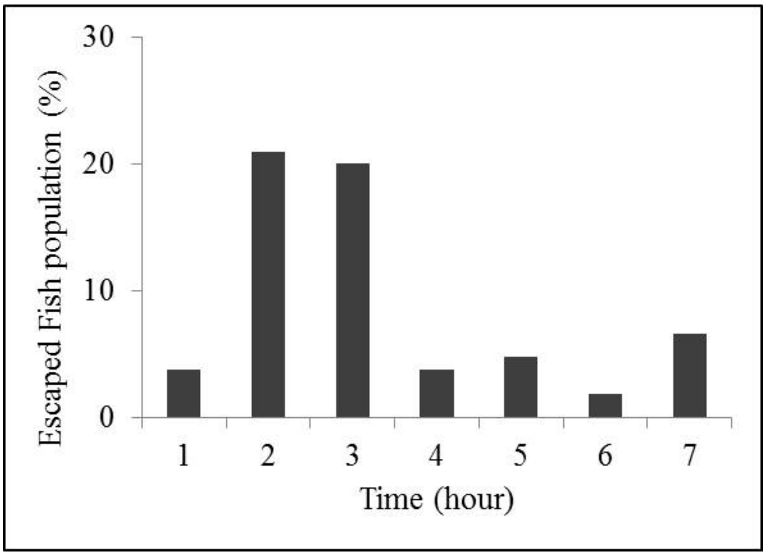

Figure 30: Percentage of fish population that escaped per hour at the external cages, in the first day of the experiment A.

Further analysis on the first experimental day, clearly demonstrated that most of the escapes (>40 %) occurred at the external cages and particularly 2-3h after the initiation of the experiment (Figure 30). No significant differences were found on the escape rate between the different hours of the day or between the three external cages ($p>0.05$). Still, the fish seemed to escape more at the first than at the latter hours of the experiment.

6.3.2. Experiment B – Presence of a net curtain at the point of escape

Sea bass individuals that were confined in the internal cages presented a similar pattern of escape, as in the experiment A, since approximately the same number of fish (55 % of the initial population) still remained into the cages by the end of the experiment.

The presence of the net curtain, 60 cm away from the point of escape, significantly reduced fish escape activity at the external cages (as compared to experiment A). This is clearly demonstrated by the fact that no significant difference ($p>0.05$) was found between the internal and external cages along the experimental days (Figure 31).

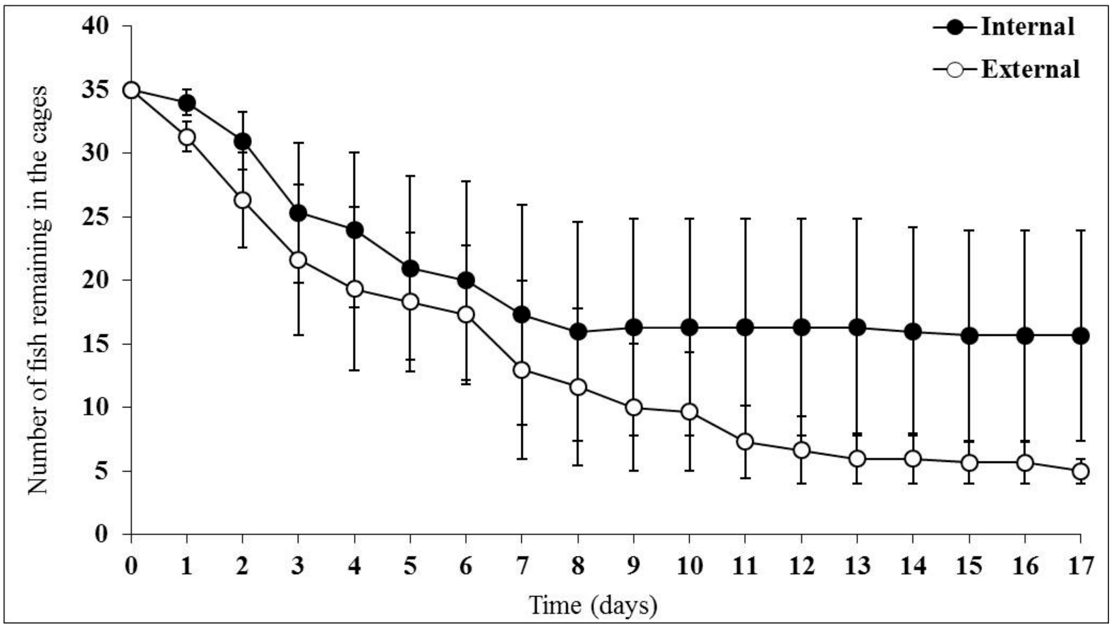

Figure 31: Number of fish (mean ± SE) remained at both internal and external cages by the end of each experimental day (17days) in Experiment B. Statistically significant differences between the two-cage groups are indicated with an asterisk.

External fish population presented higher escape activity, as in the previous experiment. However, in this case, only 10 % of the initial population escaped during the first day and approximately 23 % on the second day. By the end of day 5, most escapes occurred in one of the external cages, reflecting the 71 % of the initial population. Nevertheless, at the end of the experiment, approximately 18 % of the initial fish population still remained into the cages.

The presence of the net curtain caused a delay on the escape activity, since the first escape was observed approximately four hours after the beginning of the experiment Figure 32). Overall, the escape rate was significantly lower as compared to the one counted in the first experiment.

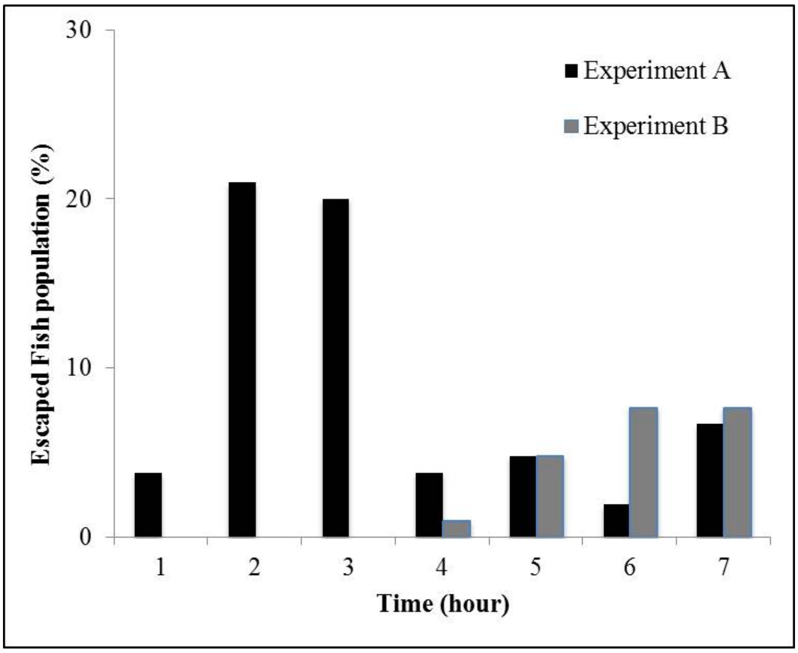

Figure 32: Comparative presentation of fish population that escaped per hour at the external cages, in the first day of the experiment A and B.

6.3.3. Differences on the escape behavior in relation to the visual conditions at the point of escape.

The percentage of the remaining fish population at the internal cages in each experiment was approximately the same, allowing the normalization of the external population by the internal one. The NRNF was lower ($p<0.001$) in the experiment A as compared to the one in the experiment B (Figure 33).

Significant differences ($p<0.05$) were observed in all the experimental days. In day 2, the NRNF that was measured in the experiment A was 8 times lower than in

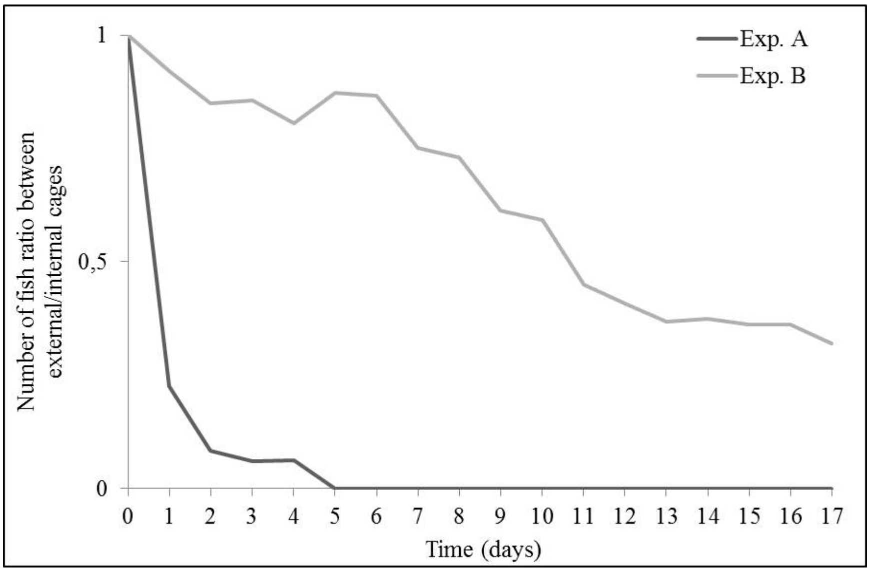

Figure 33: Proportion of the remaining fish population at the external cages by the internal ones in the Experiment A and B, over a 17-day experimental period.

the experiment B, while this difference increased up to day 5. Lastly, by the end of the experimental period, the NRNF value in the experiment A was zero since no fish remained into the external cages.

6.4. Discussion

The present study indicates that European sea bass is able to escape when a tear appeared on the net pen which comes in agreement with related studies (Arechavala-Lopez et al., 2011; Dempster et al., 2002). Furthermore, this study provides additional information on the time required for fish to escape (after a tear was located on the net pen) and the percentage of fish that escaped. The consequences of fish escaping from sea cages on the wild stocks could be detrimental (Dempster et al., 2009; Jensen et al., 2010; Naylor, 2006) and there are evidence of crossing-hybridization of some species with wild fish (Atlantic salmon) that can potentially lead to genetically alteration of the wild stocks, reduce their biodiversity and eventually affect their viability (Jensen et al., 2010). In addition, according to post-escape behavioral studies (Arechavala-Lopez et al., 2011) the European sea bass moves among several farm facilities. Its dispersal and movements could potentially lead to transfer of pathogens not only to the wild stocks but also among the different farming facilities. However no sign of pathogen transmission has been reported yet, while on the other hand, escaped fish suffer from high mortality rates, particularly because of fish predators.

6.4.1. Pre-escape and Escape behavior of *D. labrax*

Escape incidents have been largely documented from commercial farms (Arechavala-Lopez et al., 2012; Dempster et al., 2002). Nevertheless, the escape-related behavior of *D. labrax* has been occasionally documented. When held in aquaculture, sea bass presents a shoaling behavior (Malavasi et al., 2004), while no interactions are observed on the net pen. In contrast, related studies in sea bream have shown that high fish density (Papadakis et al., 2012) and food deprivation (Glaropoulos et al., 2012) increase fish interaction with the net and consequently fish propensity to escape. Thus, sea bass propensity to escape should be further investigated under different conditions. Based on the results of the present study, the visual conditions around the cage environment seem to have a clear effect on species escape behavior.

These results are consistent with those reported by (Brown, 2001), showing that fish were initially hiding or remaining motionless but gradually begun to move around, explore and colonize the new environment. By the time that *D. labrax* located the tear on the net pen; its swimming behavior was modified to initiate escapes to the outer volume of the tank. The elongate body of sea bass (Volcaert et al., 2008) and its swimming ability (Pickett & Pawson, 1994) may have contributed to the high escape rate that was measured in this study. The high escape rate of *D. labrax* that was observed in the first hours of the experiment could be explained by the initial increased fish density. Additionally, the empty outer volume of the tank may have led sea bass individuals to escape and exploit it, since they were initially reared into 40 m3 tanks, and subsequently confined to the 0.3 m3 sea cages. Related results have been obtained in sea bream study (personal data), where high fish density into the tanks caused an even higher propensity to escape. Similar responses have been observed in studies referring to fisheries management, where high propensity to escape appeared when fish were trapped by fisheries gears (Brown, 2001).

6.4.2. The effect of a visual obstacle

Differences were found on the escape rate between the internal and the external cage populations in the Experiment A, with the internal ones having a significantly lower escape rate. The main differences between the two cage groups were the presence/absence and the type of the visual obstacle at the point of escape.

In both experiments (A and B), the distance (<60 cm) between the wall of the tank and the tear on the net of the internal cages was short enough to offer fish a clear view of the obstacle. Additionally, the fish population used in these experiments was familiar to the black wall of the tank, where they have been previously reared. Thus, their unwillingness to escape could be explained by this familiarity since they already knew that they could not pass through the black wall of the tank. The above hypothesis has been also tested in other experiments (crimson spotted rainbowfish "*Melanotaenia duboulayi*"), where fish that were unfamiliar with a glass wall of tank continued their attempts to escape through the glass wall rather than the net pen. In contrast, fish familiar with the glass wall continued searching the net pen in order to locate a tear and escape (Brown, 2001). Both results clearly indicate that fish ability to cope with the new environmental challenges is associated with their escape success. This comes in agreement with other studies, where it is stated that the previous experience and knowledge of fish may have an influence on their current behavior (Berejikian et al., 2001; Coves et al., 2006); Salvanes & Braithwaite, 2006).

On the other hand, at the external cages of the experiment A, fish recognized the tear on the net presumably because of the differences in the visual homogeneity of the net pen that the tear creates. In contrast, at the external cages of the experiment B, fish were observed to accidentally locate the tear, when got very close, while they were inspecting the whole area of the net pen. They seemed to be unable to discriminate the tear on the net, possibly due to the presence of the net curtain that created the confusion effect.

6.5. Conclusion

The present study clearly indicates that the escape behavior of European sea bass is related to the visual conditions at the point of escape. The absence of any type of visible obstacle resulted into a high number of escape incidents. In contrast, at the cages where a visual obstacle existed at the point of escape, the escape rate was significantly lower.

Management on the location and orientation of the commercial sea cages should be reconsidered to prevent from large-scale events. Placing the sea bass cages between cages of other farmed species could be another implementation to mitigate the escape risk of sea bass. Additionally, the placement of an extra net pen after severe environmental conditions (storms) could minimize the escape risk of sea bass. Furthermore, it provides the required time to repair any damage that occurred in the main net pen.

7. General Discussion

Gilthead sea bream and European sea bass are the dominant farmed species in Mediterranean aquaculture. Despite a decrease in fish production in 2011, the long-term outlook for both species is still promising, following the increased expectations from the European and global fish market. Indeed, sales of both species have extended beyond the European borders, and sea bream and bass are now present in the market of Russia and USA (Figure 34).

Therefore, the industry development requires moving fish farms to more exposed areas, away from coast and protected coast locations. This, of course, brings new challenges regarding the rearing procedures and the cost in aquaculture technology, while increasing the risk of fish escape from cage farming facilities. The worldwide used technology cage aquaculture (de Azevedo-Santos et al., 2011) fits these expectations, but farmed species have to cope with unusual conditions, such as elevated stocking densities, different feeding regimes and biofouling development. Further, the surrounding environment indirectly affects the behavior of farmed fish through variations on light level and severe weather conditions that are a major cause of fish escape. A change towards even more exposed offshore aquaculture is expected in the upcoming years in connection with the increased protein demand worldwide. Therefore, fish escapes are likely to even increase in the future. Hence, it is highly necessary to address all the key risk factors that potentially lure species to cross net tears in the cage wall.


Figure 34: Farmed sea bream in public fish market

In the present study, it is clearly demonstrated that both sea bream and sea bass are able to escape, while the escape-related patterns are markedly different between the two species. Particularly for sea bream, exploratory behavior may increase the potential risk of escape through net damages. Similar behavior has been observed in Atlantic cod and has resulted in measures to prevent escapes (Moe et al., 2007). The knowledge acquired about the biological factors that lure fish to escape will help fish farmers to better understand species-specific behavioral traits and reconsider the rearing processes that in turn could reduce the escape risk. Moreover, both species are able to survive and habitat the spawn areas of the wild populations (Arechavala-Lopez et al., 2011; Arechavala-Lopez et al., 2012). Research should focus on measuring the escape risk and introducing new management tools to reduce the detrimental effects that in turn will promote a more sustainable Mediterranean aquaculture industry. All related management plans should be cost effective but also ensure the integrity of the marine ecosystem close to the aquaculture areas.

7.1. Why is it important to prevent fish to escape and what can be done?

Fish escape from offshore facilities and/or the release of fertilized eggs are very likely to take place in the aquaculture industry (Naylor & Burke, 2005). In addition, the expansion of aquaculture to even more unprotected areas increases the likelihood of escape that in turn disclose all the already known detrimental impacts to the marine ecosystem.

In Mediterranean aquaculture regulations have already been implemented to minimize the major negative consequences from fish escape, including transfer of pathogens (Rodgers and Basurco, 2009) and genetic interbreeding (Youngson et al., 2001). Sea bream and bass pathogens can be transported by human, staff and vessel in the cage facilities (Ruiz et al., 2000). Therefore, biosecurity management should be reconsidered in the fish farm industry to control and restrict the transmission of infectious diseases. Additional biosecurity programs and surveillance zones around the area of the cages have already been implemented.

The risk of fertilized eggs that potentially escape from the cages should be further investigated, and research should also focus on attempts to reduce the capacity of these released eggs to be hatched in the nature. Sterile triploids (Naylor et al., 2005) that have been proposed to mitigate the genetic pollution risk are associated with several problems. Despite the inability to reproduce, sterile individuals have lower growth performance and survival rate (Sadler et al., 2001). Moreover, marketing efforts to promote GMOs have to overcome the general public opinion (Beringer, 2000).

7.2. Sea bream and sea bass escape-related behavior

Apart from all the aforementioned mitigation measures of fish escape, the specific behavior of farmed species when confined in cages should be the first step to develop a sustainable aquaculture in Europe and worldwide. The resulting plans should take into consideration all related biological factors that motivate different species to exhibit a behavior that consequently results into net tears and escape.

The present study investigates the escape-related behavior of both sea bream and sea bass in relation to intrinsic behavior of the species and state-dependent factors relevant for Mediterranean aquaculture. The most obvious difference between the species is that sea bream regularly exhibit net interactions, whereas sea bass rarely exhibit any exploratory behavior towards the net pen.

The escape-related behavior of sea bream has been associated with the species motivation to interact with the net pen, possibly driven by search of food and exploitation of the tank volume (Papadakis et al., 2013). The above statement is in agreement with similar studies in Atlantic cod (Damsgard et al., 2012; Hansen et al., 2008; Jensen et al., 2010), a species that also exhibit exploratory behavior on the cage wall. It is evident that crucial factors, like food distribution and stocking density could affect individual behavior of farmed species in the same manner as genetic traits of these species. Particularly, net biting may be part of coping ability (Koolhaas et al., 1999) of farmed species like sea bream (Glaropoulos et al., 2012) that in nature feed on attached marine organisms in rocky habitats (Pita et al., 2002). Loose cut twines or fouling filaments might also resemble the natural prey, further increasing sea bream attraction towards the cage wall. It is evident that crucial factors, related to the rearing process (food supply, applied density, fouling presence) can affect sea bream behavior towards the net and further increase the escape risk, through net wear and tear. The aforementioned behaviors raise a significant concern for the escape risk in gilthead sea bream, considering that the biological causes for fish escape accounts for 25% of the total number of species escapees (Jensen et al., 2010). Even though one individual is not capable of quickly causing a tear, the total impact in high fish densities could represent a problem (Hansen et al., 2008).

On the contrary, confined European sea bass rarely presents distinct behavioral pattern towards a cage wall. Net interactions are rarely observed in laboratory or commercial-scale conditions but sea bass all the same quickly escape at a presence of a tear. Schooling behavior (Anras et al., 1997) is very often observed along with swimming at a distance from the cage wall, a behavior that has also been reported for Atlantic salmon (Ferno et al., 1995; Juell & Westerberg, 1993). The preference to forage in groups (Anras et al., 1997) combined with the elongated body form (Pickett & Pawson, 1994; Volcaert et al., 2008) compared to sea bream could partially explain the precise difference between sea bream and bass behavior in aquaculture. Factors like food and stocking density do not seem to influence the escape behavior of sea bass. Related studies in laboratory conditions (personal data) revealed that sea bass has a similar swimming activity in tanks regardless of the amount of food provided. Further, individuals were continuously observed to cross the net tear. However, investigation of the same factors in commercial-scale aquaculture and with larger population is yet to be done.

The comparison of the two species revealed a difference in personality trait related to social interactions. The high number of escapes (up to 7 individuals in line) in both tanks and experimental cages (Papadakis et al., 2013) indicates that sea bass under captivity act more as a group than as individuals. In contrast, almost no incident of sea bream escape was followed by another fish. This clearly demonstrates that the escape behavior of European sea bass is a mostly a state-dependent factor, where the around environment has its own significance, rather than an intrinsic individual trait of the species. This can be explained by that, sea bass mostly forage in groups in nature (Anras et al., 1997).

Sea bream individuality involving intrinsic factors, like "shyness" and "boldness" as well as social hierarchies might also affect the escape behavior. Particularly, in experiments with a net tear, sea bream did not immediately locate the tear, and it took a long time to cross the tear as compared to in experiments with sea bass. Moreover, sea bream distribution is often altered due to establishment of dominance hierarchies. Aggressive behavior of sea bream (Castro & Caballero, 2004; Cleveland & Lavalli, 2010; Goldan et al., 2003) could potentially make fish interact with the net, since establishment of territories by dominant individuals would result in less available volume for the subordinate individuals.

Overall, the present study concludes with that sea bream and bass exhibit a totally different escape pattern, while several factors, different for each species, can potentially influencing this specific behavior. Thus, it is of great importance to evaluate species motivation to damage the aquaculture net and thereby increase the possibility to form a hole and escape. As evident, management measures and handling processes of farmed fish should be reconsidered so as to mitigate the potential risk of escape and particularly of net damage due to species interactions.

7.3. Recommendations to mitigate the escape risk

It is evident that fish escapes raise a number of ecological concerns about the marine environment and thus, adequate measures should be taken into consideration to significantly reduce and/or minimize this risk. The expected growth of the aquaculture industry in the forthcoming years sets mitigations of fish escape as one of the primary objectives for a sustainable aquaculture industry. In addition, the actions taken shall be cost-effective and combine the biological background and the general behavior of the farmed species that lure them to further wear and tear the net pen.

Factors like feed distribution and applied stocking density are of great importance in modern aquaculture, since they can alter fish behavior inside a cage environment and increase the motivation to escape. From the results obtained in the present study, food-deprived fish are highly motivated to cross a net tear, probably driven by search of food. An individual moving rapidly in irregular pattern inside the cage might have a high likelihood to locate an existing net tear and escape. Therefore, feeding regimes should be reconsidered to surplus the required fish demand for energy. In addition, food distribution in the center of the cage volume would distract from net interactions and thus restrict the risk of escape from an open hole. This is in agreement with other studies, where foraging behavior dominates over exploratory behavior at the net pen (Damsgard et al., 2012; Hansen et al., 2008; Moe et al., 2007). Regular feeding period would be appropriate to reduce the escape risk in commercial –scale aquaculture.

In addition, a well-considered fish density would promote high productivity avoiding any "stress" condition to the farmed fish that may alter their behavior (Anras & Lagardere, 2004) and induce escapes. This implies that stocking density should not consider only the hydrodynamics and human activities but also fish movement inside the cage volume and potential influences due to water quality (Oppedal et al., 2011). Thus, an appropriate stocking density along with the aforementioned feeding regime should be reconsidered not only based on fish welfare and optimal production level but also on escape rate.

Moreover, the operational procedures should be based on better quality material and more adequate mooring and floating facilities, tolerant to the severe environmental conditions in the Mediterranean region. Particularly for European sea bass, appropriate installation design of the cages is highly recommended to restrict the high risk of escape at a presence of a net tear (i.e. formation, orientation). In that, the net pen of other cages around those of sea bass culture could be considered as an extra net pen around the cage environment.

Advanced technology to monitor fish activity emerge as an appropriate tool to mitigate several problems encountered in aquaculture. Direct monitoring of fish behavior (Papadakis et al., 2012) could contribute to solutions in aquaculture, by removing the need for fish handling along with minimizing human presence in the area of the cages. Moreover, special training of the staff is necessary to reduce the human involvement on fish escape during operational procedures on the cage area.

7.4. Implementations for net damage prevention

The present thesis demonstrates the level of influence that the condition of the net has on the escape-related behavior of sea bream. Similarly to Atlantic cod (Moe et al., 2007), this species frequently inspect and bite the aquaculture net and try to escape through net holes in the cage wall. In European sea bass net interactions are rarely observed in both experimental tanks and commercial cages.

From the results obtained, it is clear that sea bream mostly focus on loose threads and net repairs with escape strongly associated with feeding condition and the fish density. Thus, better net materials and frequent control of the net condition are required to avoid net damage due to interactions with the net pen. In addition, adequate feeding regimes may reduce biting in the net, since it has been shown that biting decreased during feeding time as compared to the rest of the day. Further, even though the individual force exerted to the net pen is quite low for a standard nylon twine (Hoy et al., 2012), an elevated fish density inside the cage with thousands of individuals regularly interacting with the cage wall might represent a problem for the condition of the net.

Offshore cage facilities mostly use double polyamide nets that are resistant to fish bite as well as from predators on the outside. Alternative approaches to shape the behavior of farmed species could further minimize net biting. Nevertheless, the main practical implication that ensures a low level of net interactions is that farmers should maintain net cleanliness and avoid fouling filaments developing on the net wall.

7.5. Future Research

Through this thesis it is demonstrated that several factors are correlated with species escape-related behavior. The effect of light level would have been interesting to study also in gilthead sea bream as the available light should be expected to influence net wall interactions. In addition, the relative effect of the surrounding environment on the escape rate of the sea bream has not yet been examined on a commercial scale, with fish kept under natural conditions and in higher stocking densities.

Since behavior is the first reaction to any new challenge that fish have to face, fish movement and distribution as well as social hierarchies that might develop inside the cage volume should be further evaluated in relation to fish welfare and appropriate rearing conditions. In this connection, there will be a need for advanced monitor systems. It is also crucial with regulations improvement of the current legislation that will ensure an economically efficient aquaculture industry with minimal impacts on the environment, including escapes. Lastly, risk analysis tools should be implemented to evaluate all potential factors related to fish escape.

General Conclusions

1. Identifying species-specific behavioral patterns and in detail descriptions of every discrete step through time-dependent analysis is a way to solve practical problems in aquaculture.

2. Gilthead sea bream and European sea bass present a totally different escape pattern. Sea bream frequently exhibit exploratory behavior towards the aquaculture net. In contrast, European sea bass rarely interact with the cage wall. However, its high visual acuity ensures the location of the tear from a longer distance as compared to sea bream.

3. Gilthead sea bream is able to damage the aquaculture net through exploratory behavior (inspect, nibble and bite). Sea bream focused on loose threads that release inspection and biting behaviors. Net condition thus significantly influences sea bream attraction towards the cage wall.

4. The number of escapes decreased during the feeding time in both species. The presence of food pellet seems to prevent fish from net inspection and looking for a tear. Limited-fed fish are more prone to inspect and bite the net pen, probably driven by food search.

5. In the case of light level, sea bass preferred staying in higher light intensity than return in shadow, where food was provided.

6. In contrast, the lower the fish density the higher numbers of bites occur on the net pen, which suggests that sea bream act individually and exploit the entire volume of the cage.

7. Biofouling is another risk factor for net damage. Long stranded biofouling material may act as a visual stimulus that drives exploratory behavior. Even early in the development, ie. micro-fouling, sea bream is attracted by filaments and bites the net. The number of bites decreased when no filaments exist on the net pen. Long-term biofouling presence on the net will further induce fish attraction, increasing the risk of net damage.

8. The color of the net seems to have an influence on net attraction. Research should now be focused on different colors and their influence upon sea bream interaction with the aquaculture net.

9. European sea bass usually present a strong schooling behavior, when confined in tanks or cages. Acting as group rather than as individuals, very often results into a high escape rate as well as that these escapes occur in a series of up to seven individuals.

10. Experiments also showed that the visual conditions around the cage area have an effect on large-scale sea bass escapes. Particularly, the presence of any visible/solid obstacle at the point of escape significantly reduces the escape risk of European sea bass.

11. Biotic and abiotic factors have been associated with the escape-related behavior of both species. Food supply, stocking density seem to have no effect on sea bass escape behavior, when this is tested in experimental tanks, probably due to the general biology and behavior of the species. The effect of light should be furthermore investigated under commercial scale aquaculture, when variations on light intensity in close related to the general environmental conditions in the farming area.

Figures and Tables

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Paper Related to this PhD thesis

1. **Alexios Glaropoulos**, Vassilis M. Papadakis, Ioannis E. Papadakis, Maroudio Kentouri (2012). Escape-related behavior and coping ability of sea bream due to food supply. *Aquaculture International*, 20: 965-979.

2. Vassilis M. Papadakis, Ioannis E. Papadakis, Fani Lamprianidou, **Alexios Glaropoulos**, Maroudio Kentouri (2012). A computer-vision system and methodology for the analysis of fish behavior. *Aquacultural Engineering*, **46**: 53-59.

3. I. E. Papadakis, V. M. Papadakis, **A. Glaropoulos**, S. I. Petroutsos, L. Png. Gonzalez, M. Kentouri (2013). Do the visual conditions at the point of escape affect European sea bass escape behavior? *Mediterranean Marine Science*, **14 (1)**: 172-178.

4. Ioannis E. Papadakis, Vassilis M. Papadakis, **Alexios Glaropoulos**, Fani Lamprianidou, Maroudio Kentouri (2013). Escape-related behavior of juvenile gilthead sea bream (*Sparus aurata*) versus rearing density in experimental conditions. *Journal of Biological Research – Thessaloniki*, **20**: 208-216.

5. **Alexios Glaropoulos**, Vassilis M. Papadakis, Ioannis E. Papadakis, Antonia Georgara, Maroudio Kentouri (2014). Sea bream interactions towards the aquaculture net due to the presence of micro-fouling. *Aquaculture International*, **22**: 1203-1214.

6. **Alexios Glaropoulos**, Vassilis M. Papadakis, Eleftheria Georgiou, Ioannis E. Papadakis, Maroudio Kentouri (…). Different light level affects the escape response of European sea bass. *Aquaculture Research*, under review.

7. Vassilis M. Papadakis, **Glaropoulos Alexios**, Maroudio Kentouri (...). Time-dependent analysis of fish behavior using a novel computer-vision system. *Aquacultural Engineering*, under review.

Conference Proceedings

1. **A. Glaropoulos**, V.M. Papadakis, I.E. Papadakis and M. Kentouri, “Computer vision aided system for behavioral analysis of fish.“ in Proceedings on 5th International Congress on Aquaculture, Fisheries Technology and Environmental Management, AquaMedit, November 2010, Messolonghi, Greece.

2. IE. Papadakis, F. Lamprianidou, VM. Papadakis, **A. Glaropoulos**, M. Kentouri, "The escape behavior of sea bream (*Sparus aurata* L.) in relation with the stocking density." in Proceedings on 5th International Congress on Aquaculture, Fisheries Technology and Environmental Management, AquaMedit, November 2010, Messolonghi, Greece.

3. I.E. Papadakis, V.M. Papadakis, A. Georgara, **A. Glaropoulos**, M. Kentouri, "The effect of the biofouling presence on the behavior of *Sparus aurata* towards the aquaculture net." in Proceedings on the European Aquaculture Society Congress, 18-21 / October, 2011, Rhodes, Greece.

4. VM. Papadakis, IE. Papadakis, **A. Glaropoulos**, M. Kentouri, "A Computer Vision System for the analysis of Fish Behavior." in Proceedings on the European Aquaculture Society Congress, 18-21 / October, 2011, Rhodes, Greece.

5. **A. Glaropoulos**, I.E. Papadakis, V.M. Papadakis, M. Kentouri, "Escape behavior and coping ability of Sparus aurata due to food supply." in Proceedings on the European Aquaculture Society Congress, 18-21 / October, 2011, Rhodes, Greece.

6. I.E. Papadakis, V.M. Papadakis, S.I. Petroutsos, **A. Glaropoulos**, M. Kentouri, "Evaluation of escape behavior and learning ability of *Dicentrarchus labrax*." in Proceedings on the European Aquaculture Society Congress, 18-21 / October, 2011, Rhodes, Greece.

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12. V.M. Papadakis, A. Antonakaki, **A. Glaropoulos**, M. Kentouri, "A Methodology for the Analysis of sea bream Larvae Behavior in Aquaculture." In Proceedings on Aquaculture conference: To the Next 40 Years of Sustainable Global Aquaculture, 03-07/11, 2013, Gran Canaria, Spain.

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This certifies that

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have been selected to receive an Aquacultural Engineering Society Honorable Mention Paper Award given in recognition of authorship of a contribution to aquacultural engineering literature of exceptional merit.

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