Mediterranean Marine Science

Vol 14, No 1 (2013)

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doi: 10.12681/mms.360

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
http://dx.doi.org/10.12681/mms.360

Do the visual conditions at the point of escape affect European sea bass escape behaviour?

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Received: 28 September 2012; Accepted: 1 February 2013; Published on line: 22 March 2013

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 create tears. Escapees may interact with wild stocks through interbreeding, transfer of pathogens and competition for food. The aim of this study was to examine to what extent the presence of a visible obstacle close to a tear in the net influences sea bass propensity to escape. Fish were initially confined into small sea cages, with a tear on one side. The escape behaviour was tested under experimental conditions. It is clearly demonstrated that sea bass were able to locate a tear in 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 behaviour of *D. labrax*, resulting in a delay that eventually reduced the escape rate. To conclude, 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.

Keywords: Cage aquaculture, escape, European sea bass, behaviour, obstacle, net pen.

Introduction

Escapes of fish from sea cage installations raise a number of remarkable concerns for 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 threaten 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. A company's reputation as well as conflict with environmental groups is considered as the most important consequence (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 *et al.,* 2005).

Such escapes have been reported for almost all species that are reared in European aquaculture (*Sparus aurata*, *Dicentrarchus labrax*, *S. salar*, *G. morhua*) installations and occur at all the stages of the rearing process (Haffray *et al.,* 2007), such as 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 escapes have been reported after intense coastal storms in Norway (Norwegian Fisheries Directorate, 2007). Fish predators attacking the cages may also create holes in the cage net (Dempster *et al.,* 2002; Jensen *et al.,* 2010). Recently, a significant number of studies have been focussed on the pre-escape behaviour (fish inspection and biting the net pen) of the farmed species, since it may lead to damages in the net pen and the 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 of European aquaculture, which regularly present the above specific behaviour.

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) as soon as a tear appeared in the net pen. No interaction with the aquaculture net pen has been documented, and sea bass does not thus 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 in the net and escape.

The aim of this study was to evaluate the escape behaviour 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 a tear affects the propensity of fish to escape. Also, we tried to evaluate to what extent the type of obstacle influenced this specific behaviour.

Materials and Methods

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. The larval rearing process, until fish metamorphosis, was performed using mesocosm technology (Divanach & Kentouri, 2000) in 40 m^3 tanks. After that, fish were transferred to 10m^3 pre-growing tanks, where they were kept for 150 days, under the same rearing conditions. A total 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).

Sea cage preparation

The experiments were performed in six handmade sea cages (length 60 cm, depth 80 cm and width 60 cm) that were placed in a 17 m^3 semicircle tank. Common white aquaculture net was used for the sea cages. A tear was created on one side of the net; large enough (5cm) for fish to escape. Natural sea water renewal was ensured by a constant flow (10%/h). The temperature was 20°C, the salinity was 38‰ and dissolved oxygen was above 95%, throughout the whole experimental period. The experiment was conducted under a natural photoperiod (daylight: from 06:00 a.m. to 08:00 p.m.).

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 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 1.8m and it was equal for the two cage groups.

Thirty-five (35) fish individuals were initially confined to each one of the six sea cages. Food was supplied once per day, equally for all six cages (2% of the initial total body weight of the fish population per cage). Food was also administered manually and in small amounts, inside the cage environment, to give the fish sufficient 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 of the internal cages was facing the front black wall of the tank, while the tear of the external cages was facing the open view volume of the tank. In the 2nd experiment, a net curtain (length: 3m, width: 2.2m) 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 like the wall of the tank for the internal group.

Monitoring fish activity in the cages

Six external cameras that were installed above the cages, each recording a single cage, allowed observation of fish activity in the sea cages. The cameras were all connected to a computer, located outside the experimental area, thus eliminating the influence of human presence on fish behaviour. Acquisition was performed through a multi-camera frame grabber (GV-1120, Geovision), able to record video data from all cameras simultaneously. 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., when natural light variations were acceptable. Then, the video stopped and started again the next day. Data for each day were transferred to the computer and stored as avi file.

Data extraction and analysis

All acquired video data were visually analysed, using 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 i.e. the number of incidents where one fish crossed the tear to the outer space of the cage. The exact time of each incident was also recorded so as to provide detailed information on the escape behaviour of *D. labrax* individuals. Finally, the remaining fish population in the cages was counted at the end of each experimental day so as to provide the overall remaining 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 at the end of each day. Then, the normalized remaining number of fish (external/internal), referred to as NRNF, was calculated for each experiment, dividing the external population by the internal one, for each experiment.

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.

For the normal distribution data, the differences between the groups were detected using the Student–Newman–Keuls test; for data without a normal distribution, nonparametric control Kruskal–Wallis and Mann–Whitney tests were applied. Data were treated groupwise (n = 3). The level of significance was set at 5% ($p<0.05$). The standard error was calculated for all the mean values.

Results

No incident of mortality or cannibalism was observed during the experiments. Sea bass individuals appeared to be motionless at the time of confinement to 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 in the number of escapes that occurred were associated with the visual conditions at the point of escape. No differences were found ($P > 0.05$) between the replicates in any of the experiments.

Experiment A - Solid visual obstacle at the point of escape

The presence of the tank wall (solid obstacle) resulted in a lower ($p<0.05$) escape activity between the two cage groups. As regards the external cages, approximately 80% of the 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, for the internal cages, only 6% of the initial fish population had escaped on the first day. Furthermore, approximately half (50%) of their initial population still remained in the cages by the end of the experiment (Fig. 1).

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

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

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

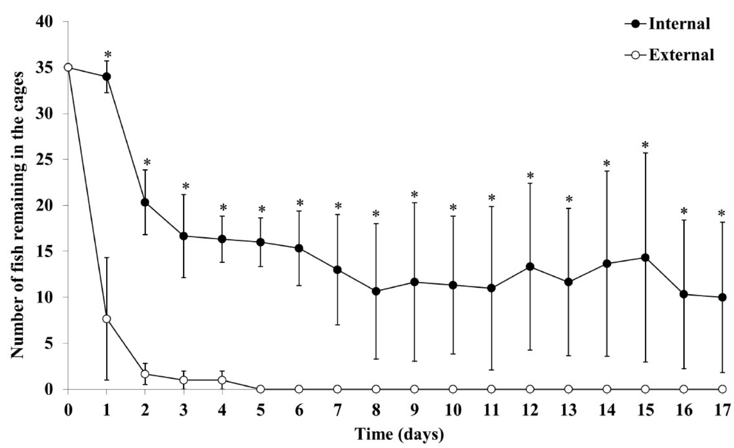

Fig. 1: Number of fish (mean ± SE) remaining in both the internal and external cages by the end of each experimental day (17days) in Experiment A. Statistically significant differences between the two-cage groups are indicated with an asterisk.

end of the experiment.The presence of the net curtain, at a distance of 60cm from the point of escape, significantly reduced fish escape activity in 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 (Fig. 3). The 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, corresponding to 71% of the initial population. Nevertheless, at the end of the experiment, approximately 18% of the initial fish population still remained in the cages.

The presence of the net curtain caused a delay in escape activity, since the first escape was observed approximately four hours after the beginning of the experiment (Fig. 2). Overall, the escape rate was significantly lower as compared to the one computed for the first experiment.

Differences in escape behaviour in relation to the visual conditions at the point of escape

For each experiment, the percentage of the remaining fish population in the internal cages was approximately the same, thus allowing the normalization of the external population by the internal one. The NRNF was lower ($p<0.001$) in experiment A as compared to the one in experiment B (Fig. 4).

Significant differences ($p<0.05$) were observed on all the experimental days. On day 2, the NRNF measured for experiment A was 8 times lower than for experiment B, while this difference increased up to day 5. Lastly, by the end of the experimental period, the NRNF value for experiment A was zero since no fish remained in the external cages.

Discussion

This study indicates that European sea bass is able to escape when a tear appears in the net pen, which is 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 in 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 is evidence of crossing-hybridization of some species with wild fish (Atlantic salmon) that can potentially lead to genetic alteration of wild stocks, reduce their biodiversity and eventually affect their viability (Jensen *et al.,* 2010). In addition, according to post-escape behavioural studies (Arechavala-Lopez *et al.,* 2011) the European sea bass moves between several farm facilities. Fish dispersal and movements could potentially lead to the transfer of pathogens not only to the wild stocks but also the various farming facilities. However, no indication of pathogen transmission hav been reported yet, while on the other hand, escaped fish suffer from high mortality rates, particularly because of fish predators.

Pre-escape and Escape behaviour 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 behaviour of *D. labrax* has only been documented occasionally. When kept at aquaculture facilities, sea bass present a shoaling behaviour (Malavasi *et al.,* 2004), while no interactions with the net pen are observed. In contrast, related studies on sea bream have shown that high fish density (Papadakis *et al.,* 2012) and food deprivation (Glaropoulos *et al.,* 2012) increase fish interaction

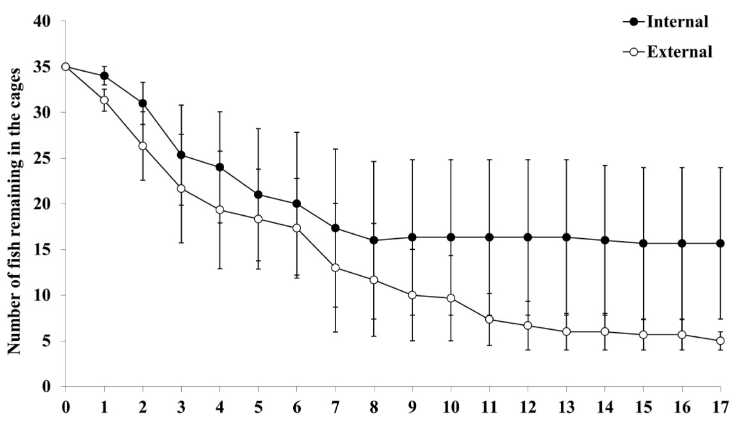

Fig. 2: Percentage of fish population that escaped per hour from the external cages, on the first day of experiments A and B.

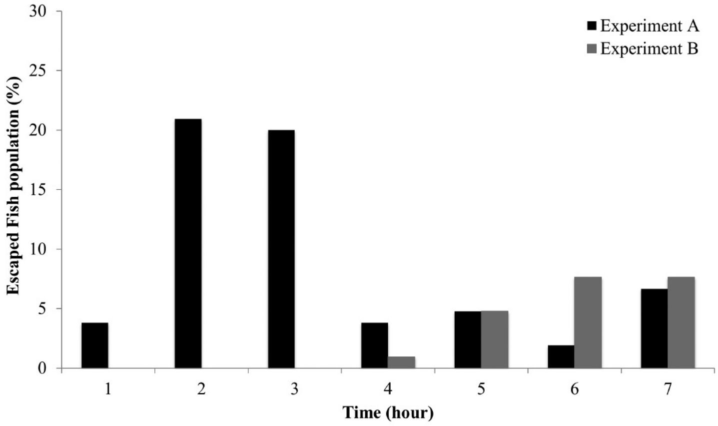

Fig. 3: Number of fish (mean ± SE) remaining in both the internal and external cages by the end of each experimental day (17days) - Experiment B. Statistically significant differences between the two-cage groups are indicated with an asterisk.

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 this study, the visual conditions around the cage environment seem to have a clear effect on species escape behaviour.

These results are consistent with those reported by Brown (2001), showing that fish initially hide or remain motionless but gradually begin to move around, explore and colonize the new environment. As soon as *D. labrax* located the tear on the net pen, swimming behaviour changed in order to initiate escapes to the outer volume of the tank. The elongated 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 40m^3 tanks, and subsequently confined to the 0.3 m^3 sea cages. Related results have been obtained in sea bream study (personal data), where high fish density in the tanks caused an even higher propensity to escape. Similar responses have been observed in studies referring to fisheries management, where a high propensity to escape was observed when fish were trapped by fisheries gears (Brown, 2001).

The effect of a visual obstacle

Differences were found in the escape rate between the internal and the external cage populations of Experiment A, with the internal ones having a significantly

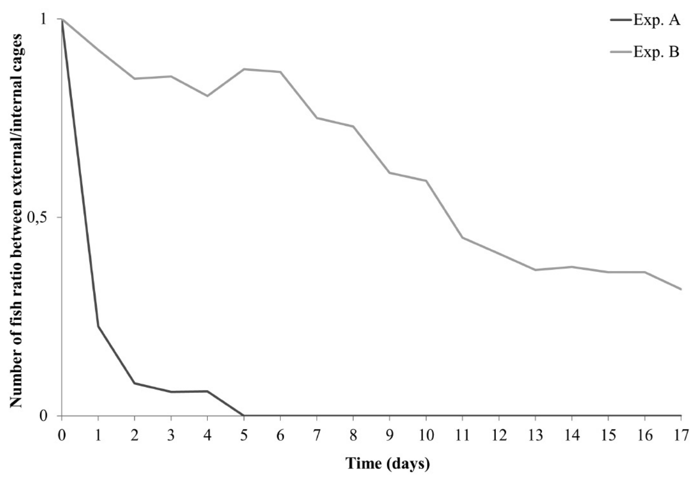

Fig. 4: Proportion of the remaining fish population in the external cages by the internal ones in the Experiment A and B, over a 17-day experimental period.

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 in the net of the internal cages was short enough to offer the fish a clear view of the obstacle. Additionally, the fish population used in these experiments was familiar with the black wall of the tank, where they had been reared previously. 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 also been tested in other experiments (crimson spotted rainbowfish *Melanotaenia duboulayi*), where fish that were unfamiliar with a glass tank wall continued their attempts to escape through the glass wall rather than the net pen. In contrast, fish that were 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 the ability of fish to cope with the new environmental challenges is associated with their escape success. This is in agreement with other studies, where it is stated that the previous experience and knowledge of fish may have an influence on their current behaviour (Berejikian *et al.,* 2001; Coves *et al.,* 2006; Salvanes & Braithwaite, 2006).

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

Conclusion

This study clearly indicates that the escape behaviour of European sea bass is related to the visual conditions at the point of escape. The absence of any type of visible obstacle resulted in a high number of escape incidents. In contrast, for the cages with a visual obstacle at the point of escape, the escape rate was significantly lower.

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

Acknowledgements

This study has been financially supported by the Commission of the European Communities under the Seventh Research Framework Programme Food, Agriculture and Fisheries and Biotechnology, Area 2.1.2 Increased sustainability of all production systems (agriculture, forestry, fisheries and aquaculture); plant health and crop protection”, 226885 ”PREVENT ESCAPE - Assessing the causes and developing measures to prevent the escape of fish from sea cage aquaculture“. The authors would like to thank the Hellenic Centre for Marine Research (HCMR) and especially its director, Dr. Pascal Divanach for providing the sea bass and the facilities to perform our experiments.

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