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Performance of the cultured Mediterranean mussel *Mytilus galloprovincialis* (Lamark 1819) after summer post-harvest re-immersion

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Abstract

Post-harvesting, handling and storage practices, particularly during the summer months, are crucial for mussel farms, as they induce stress, mortalities and affect overall performance and marketability. In the current work, Mediterranean farmed mussels, were stored into plastic net bags of different weights (7.5kg, 10 kg, 12.5 kg and 15 kg) and re-immersed into the farm sea water during summer 2012. The experiment was conducted under warm summer conditions in Mediterranean waters with temperature levels close to the upper tolerance limits of the mussels (28ºC). Mortality rates and condition index (CI) were calculated in all mussel bags at four sampling dates over a 46 days period. It is concluded that mussels graded-packed on bags (7.5-15 kg) could be preserved underwater for 25 days of re-immersion without significant mortality and CI losses.

Introduction

Mussel mortality particularly during summer months with increasing water temperature and decreasing dissolved oxygen, is of great concern among farmers. This could lead to considerable losses of the bivalve stock, characterised as mass mortalities (>30%) in farms located in bays with limited water circulation and/or when algal blooms are forming (Guillotreau *et al*., 2017). Moreover, one of the most important causes of harvested mussel mortality is the 'artificial' stress, (i.e. inflicted by human interference) during practices such as de-clumping, washing, grading and de-byssing. Among these practices, grading is recognised as one of the greatest causes of such stress for the commercial farmed bivalves such as Mediterranean mussels (*Mytilus galloprovincialis,* Lamarck, 1819) and pacific oysters (*Crassostrea gigas*

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Thunberg, 1793) (Zhao, Li, Luo, & Chang, 2011). It has also been observed that Neutral Red retention times decrease with increasing time spent being graded (Zhang, Li, Vandepeer, & Zhao, 2006).

In the Mediterranean countries, including Greece, it is common practice to re-immerse harvested and graded mussels into the seawater for several days before being transported to major European markets such as Brussels, Madrid, Paris and Rome (Angelidis, 2007). Harding, Couturiera, Parsons, & Ross (2004a) investigated the stress response of the Atlantic mussels (*Mytilus edulis* L.) in reaction to post-harvest conditions during handling, processing and storage practices, based on lysosomal stability. They concluded that there may be a reduction in stress response, better product quality and longer shelf life if a 24-hour re-immersion period is applied before transportation. Shelf life is defined as the time elapsed before a predetermined percentage of shellfish have died. In the case of mussels, mortality ≥10% in bags may produce odours associated with microbial growth, deterring the consumers from the product. For *M. edulis* farming industry, the end of shelf life is stricter as it was established at 5% mortality (Hardin *et al*., 2004a; Harding, Couturier, Parsons, & Ross, 2004b). Short term re-immersion has also been proven to improve the strength and ability of the Mediterranean mussels to deal with the subsequent transportation stress (Angelidis, 2007; Theodorou, Viaene, Sorgeloos, & Tzovenis, 2011). Moreover, survival of mussels during the transportation period is basically relied upon maintaining the cooling chain. Extensive studies on the Mediterranean mussels (Angelidis, 2007) have shown the importance of maintaining an optimum temperature at a range of 0-5°C, in order to prolong significantly the shelf life of the product exposed to air.

Re-immersion method was introduced in Greece in the early 1990's in order to overcome the harvesting bans due to harmful algal blooms (HABs) that has been challenging the economic sustainability of the mussel farming industry (Economou et al., 2007). Within the wider context of climate change and sea warming, heat waves are more frequent from year to year (Coma *et al*., 2009; Fischer, & Schär, 2010; Rodrigues *et al*., 2015; Galli, Solidoro, & Lovato, 2017) during the harvesting period of the product in Greece (i.e. summer months). Given the fact that only a part of the harvested quantities is shipped immediately to the markets, mussels are frequently stored after grading in the sea at water temperatures around 28-30ºC, close to their tolerance limits (Anestis, Lazou, Pörtner, & Michaelidis, 2007; Anestis *et al*., 2010; Ioannou, Anestis, Pörtner, & Michaelidis, 2009; Anestis, Pörtner, & Michaelidis, 2010). Moreover, the range expansion of invasive non-indigenous biofouling ascidians such as the light-bulb sea squirt *Clavelina lepadiformis*, reduce the available space for mussels, consume oxygen and require further grading and handling efforts (Centroducati *et al*., 2006). The aim of this preliminary field work was to investigate the effects of various underwater storage periods and mussel bag stocking densities during summer water conditions in central Greece, on key parameters such as mussel mortality and condition index (CI).

Materials and Methods

Study Area

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Field experiments took place in a coastal long line mussel farm (at depth of 9 m) in Molos coastal area of the southern part of Maliakos Gulf (Figure 1). This gulf is an Aegean Sea semi-enclosed embayment covering 110 km². It is situated on the east coast of continental Greece (38°51'39.82" N, 22°41'45.54" E). The gulf and particularly the study site receive significant nutrient inputs from the River Spercheios (Kormas, Kapiris, Thessalou-Legaki, & Nicolaidou, 1998; Markogianni, Varkitzi, Pagou, Pavlidou, & Dimitriou, 2017). Salinity ranges from 20 to 36 ppt and according to primary production and nutrient concentrations recorded, it is classified as a eutrophic marine ecosystem (Markogianni *et al*., 2017).

The gulf is a natural habitat of a wide range of bivalve species stocks. The natural recruitment of the Mediterranean mussel in this area was enough for further exploitation through extensive farming (Chatzonikolakis *et al*., 2017). A cluster of several long line mussel farms was recently established (Theodorou *et al*., 2011; Theodorou, Perdikaris, & Filippopoulos, 2015). The entire area was recognised as protected within the EU Natura 2000 Network and mussel farms are considered as an environmentally compatible activity (Neofitou *et al*., 2014; Dimitriou *et al*., 2015). In fact, mussel farming zones are integral parts of the nationwide spatial planning for the aquaculture sector (i.e. the Common Spatial Planning Framework for Aquaculture ratified on 4 November 2011 by the Common Ministerial Decision No 31722/2011, FEK 2505). According to this framework, Maliakos Gulf belongs to the particularly developed areas from the aquaculture viewpoint, and it is currently in the final stage of zoning declarations via Presidential Decrees.

Experimental Method/Sampling

Mussels of marketable size (>5.5-6cm) were harvested from *pergolaris* - type bunches (i.e. mussel "socks" made of plastic cylindrical nets, allowing mussels to aggregate and therefore to be handled by hand) on the 1st of July 2012. Subsequently, they were manually de-clumped, graded and placed into sixteen identically sized plastic netting bags (20 mm mesh size) with total weights of 7.5 kg, 10 kg, 12.5 kg and 15 kg (four bags per weight size) (Figure 2). All bags were marked with small bits of string, attached to the same 'mother' line, approximately 40 cm apart and immersed in the mussel farm at 60 cm below the surface. The bags (one per different weight) were collected on days 11 (11th of July-D11), 25 (25th of July-D25), 37 (7th of August-D37) and 46 (16th of August-D46) after immersion (D0) for condition index and mortality estimations. Seawater temperature was recorded *in situ* and air temperature was provided by the reports of the closest National Meteorological Station at Ano Vardates in Lamia (Lat: 38.8, Lon: 22.42; Altitude: 458 m) from 1st of June until 10th of September 2012. Data relevant to the dissolved oxygen, water temperature and transparency of the Maliakos Gulf for the same period were mined in the datasets of the Fthiotida Regional Department of Fisheries.

Data Analysis

The weight of each bag was measured using a hanging scale (kg). The bags were then opened and 15 and 10 externally and internally positioned mussels were selected randomly for calculations. External mussels

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made up the very outer layer of the bag. Internal mussels were defined as ones that were at least 5 cm inside the bag. It was possible to do this task as once re-immersed, the mussels start to join together using their byssus threads. This enabled the bag to stand alone as a ball of mussels when the bag was removed. The number of dead and alive animals obtained from the bag was then counted (as pooled samples of internal and external layers) and mortality was calculated over the respective immersion period. Mussels were determined dead when open with no ability to close their shell, even when tapped. Total volume (ml) of each animal was measured using 500 ml measuring cylinder. Subsequently, each individual mussel was opened using a scalpel and shell and wet meat components were separated. Condition Index (CI %) was calculated volumetrically, using 20 ml volumetric tube and following the procedure developed by Baird (1958). This formula is the simplest and easiest way to work in the field with live animals and it was previously applied in a similar study to Atlantic mussels by Lutz, Incze, Porter and Stotz (1980) as follows:

CI % = [Volume of flesh (ml) / Volume of shell cavity (ml)] X 100.

This formula compares the volume of the freshly removed tissues (measured by the displacement of water in a graduated vessel) with the volume of the shell cavity (measured as the difference in displacement between the whole animal (valves closed) and the shucked valves alone (soft tissues removed).

The mortality rate (M) in each bag, as proportion of dead animal to total animals of each bag, was estimated. The mortality rate per time space of each bag-size set (Mp) by follow formula was estimated:

$Mp_{i,j}=M_{i,j}-M_{i-1,j}$, where,

i the time (i=1 for time space D0 to D11, i=2 for time space D11 to D25, i=3 for time D25 to D37 and i=4 for time D37 to D46), j the bag (j=1 for bag 7.5kg, j=2 for bag 10kg, j=3 for bag 12,5kg and j=3 for bag 15kg).

Statistical comparisons were performed using multifactor ANOVA (Multifactor ANOVA) with Tukey's HSD test for *post hoc* analysis for a) CI between bag size, sampling day and location of mussels in bag (external-internal) and b) mussels mortality rate per time between bag size and time (Multifactor ANOVA; f-ratio; P=0.1, Tukey's HSD test; P=0.1) (Zar, 1999).

Results

Water temperature (Figure 3a) was measured at levels above 20ºC throughout the summer months (from June until mid September 2012) and above 26.6ºC during the sampling period. The maximum air temperature (Figure 3a) ranged between 26 and 39ºC with numerous peaks which coincided with the sampling dates. Dissolved oxygen levels never fell below 5.3 mg l^{-1} (Figure 3b), although it followed a slow decreasing pattern from 1st of June onwards. Finally, transparency ranged between 3-5.3 m (Figure 3c).

Mussel mortality (Mp) (%) in each different bag weight for the re-immersion period is shown on Figure 4. An obvious extreme increase of Mp after the period D25 to D37 (Mp>0.3) in bag size 15 kg than other bag size sets (Mp<0.2) was showed (Figure 4A) while Mp was not differed significant among the bag size and time (MANOVA; f=2.07, df=6,14; P=0.16). After exception of analysis the bag size 15 kg, statistically

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significant difference of Mp between the time spaces was showed (MANOVA; f=14.7, df=3,10; P=0.006), while the Mp wasn't differed between the bag size (MANOVA; f=0.21, df=2,10; P=0.81) (Table 1). The Tukey's HSD test indicated that the Mp during the time D0-D11 and D25-D37 was statistically significant greater (Mp>0.067) than the Mp during the time D11-D25 and D37-D46 (Mp<0.03) (Figure 4B). The cumulative mortality (Mc) at the finish of experimental (D46) for bag size 15 kg was 0.78 while for the others bag sizes was lower than 0.21 (Figure 4A).

The multifactor ANOVA showed that the statistically significant main factors on condition index were the sampling day (MANOVA; f=22.48, df=4,605; P<0.01) and the location of mussel in bag (internal-external) (MANOVA; f=2.74, df=1,605; P<0.1), while from the interactions, was the sampling day - bag size (MANOVA; f=2.19, df=12,605; P<0.01) (Table 2). The Tukey's HSD test indicated that the CI's of D0 and D25 were statistically significant greater than CI's of D11, D37 and D46 (Fig.5A). Also, the CI of mussels located external to clump in bag was statistical significant greater than them in internal (Fig.5B). Finally, the CI of bag-size 15 kg and of bag-size 12.5 kg were statistically significant greater than other during the D11, D25 and during the D37, respectively (Fig.5C).

Discussion

Based on the current field results, mussels' mortality increased on the first 11 days of re-immersion for all the examined bag sizes. This can be explained from the death of the "highly stressed" and "injured" animals after the grading process. After that, mortality is remaining low in all the examined bags, up to day 25 which is proposed as the maximum underwater storage period, prior harvesting. This is a highly suitable time for short period post-harvesting storage enabling the farm to cope with logistic management that can be highly crucial, especially during harvesting bans. Mussels in 15 kg bags shows significant higher mortality rates than the rest of categories from D25 to D46 and are not suitable for such as long periods of underwater storage.

The Condition Index preserved as the same in all the examined bags up to 25 Days re-immersion. The early dropped values of CI on D0-D11 could be occurred due to the increase of the water transparency (Figure 3) which is usually related with the decrease of turbidity. This is in accordance with Irisarri, Fernández-Reiriz and Labarta (2015) who shows that the temporal decrease of the turbidity which is related with the dilution of the suspended particulate organic matter (POM) (increase the transparency) reflected to short term reductions of the CI as well as on the biochemical composition (proteins and lipids reserves) of the mussels. In addition, the temporal increases in turbidity did not seem to significantly affect bivalves' proximate composition and meat yield over a longer time scale.

The observed increase in CI from D11 to D25 could be attributed to a temporary recover of the mussels after the handling and grading shock of the mussels during D0. This corresponds with the findings of Wyatt et al. (2013), for the blue mussel *M. edulis*, for which they recommend a maximum of one-month storage. Overall, an increase in condition index can be caused by an increased fluid accumulation in bivalves, due to physiological imbalance. Therefore, an increase in CI may mean either a "recovery" or it may mean also a

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negative impact on the mussel physiology. Nevertheless, fast assessment of CI by the 'dry' method (Wyatt *et al*., 2013; Gallardi *et al*., 2014) in farm conditions is more time consuming compared to method applied in the current work.

CI was influenced by the position of the mussels within the bag. The CI of the external mussels within each bag is higher than that of the internals. A possible explanation for these results is that external mussels were fed better than internals and subjected to less mechanical pressure from the bivalves' aggregation in the inner part of the netting bag.

At present, the traditional mussel farms in Greece that produce and sell for the local market, grade all their mussels manually in open air conditions. This is not only labour intensive, but also can take up to six hours to grade enough mussels by five persons in order to produce 1.5 tons of ready to market mussels. One person needs to shovel the mussels onto the grading table, two to grade and two to pick out the dead mussels, remove the sea squirts and fill the bags with graded mussels. Therefore, having this in mind, some mussels will be waiting to be graded on board, usually in piles or in buckets, for up to six hours. The reasons behind the preference of the traditional practice of hand-grading is that mechanical grading reduces the market value of the final product and it is considered as efficient when: a) the product has the highest meat content and the hardest possible shells and b) the product is going directly for processing (i.e. meat separation, steaming); otherwise the mussels are cracked without being visible during the grading inspection and strong post-harvesting complaints have been recorded from the rest of the market chain, wholesalers and retailers.

This extended handling period is directly related to greater exposure to stress, both 'artificial' and environmental such as temperature increases, which may lead to desiccation (Zhang *et al*., 2006; Zhao *et al*., 2011). Zhang and Li (2006) also demonstrated that even for a short grading period (just nine minutes), pacific oysters showed symptoms of stress response. Bivalves when exposed to the air, they open and close their valves (gaping) periodically to maintain the oxygen flow through the tissues ensuring a marginal renewal of oxygen (Guderley, Demers, & Couture, 1994). This gaping behaviour is further induced by the ambient Mediterranean region temperatures (around 20ºC). It is demonstrated that frequent opening of the valves promotes the releasing of the intra-valve water, enhance the metabolic damage of the gill tissue, sharply increase the carbon dioxide and ammonia concentrations (that could lead to acidosis under certain circumstances) and decrease the oxygen uptake up to 87% (Angelidis, 2007). Although spat of *M. edulis* showed reduced contribution (11%) of anaerobiosis towards the total energy metabolism compared to adults (>50%) in hypoxia, the time for induction of significant anaerobic component declined from ca 42 h at 4.8 kPa to 5 h at 1.0 kPa (Wang, & Widdows, 1993). Therefore, prolonged air exposure during mussel handling justifies the application of short re-immersion periods (particularly in higher depths when water temperature is high). For bigger productions scales the farms should also assess the possibility of using faster grading methods or ideally invest in using a 'French type' grading machine (Theodorou *et al*., 2014; 2015), which can grade up to 3000 kg h^{-1} and accordingly reduce handling time.

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Conclusions

The current work demonstrated the importance of re-immersion as a valuable practice for mussel post-harvesting handling, even in harsh conditions of high summer water temperatures. Mussel bags of different weights (7.5 to 15 kg) could be stored underwater up to a period of 25 Days without significant losses and CI subgrades. However, prolonged re-immersion produced sharply increased mortalities in 15 kg bags and sub-value the CI in all the bag ranges. Therefore, a result-based guideline can be given for wet storage, at a maximum of 25 days during the summer months when water temperatures are at their highest.

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Table 1. Results of multifactor ANOVA on the mussel mortality per time. SS: Sum of squares, Df: degrees of freedom, MSQ: mean square, %ExplVar: % explained variance.

FACTOR	SS ($x10^{-3}$)	Df	MSQ SS ($x10^{-3}$)	F-Ratio	P-Value	%ExplVar
Time	7.62	3	2.54	14.7	0.006	86.9
Bag size	0.0073	2	0.036	0.21	0.81	0.8
Residual	0.86	5	0.17			
Total SS	8.77	10				

Table 2. Results of multifactor ANOVA on the mussel condition index. SS: Sum of squares, Df: degrees of freedom, MSQ: mean square, %ExplVar: % explained variance.

Factors Main Effects	Ss	Df	Msq	F-Ratio	P-Value	%Explvar
A: Sampling day	2730.6	4	682.7	22.48	0.000	12.7
B: Location	84.7	1	84.7	2.74	0.098	0.4
C: Bag size	52.1	3	17.4	0.56	0.640	0.2
Factors Interaction	SS	Df	MSQ	F-Ratio	P-Value	%ExplVar
A x B	57.1	4	14.3	0.46	0.758	0.3
A x C	811.9	12	67.7	2.19	0.001	3.8
B x C	20.0	3	6.7	0.22	0.719	0.1
A x B x C	161.1	12	13.4	0.43	0.724	0.8
Residual	17488.5	566	30.9			
Total SS	21453.3	605				

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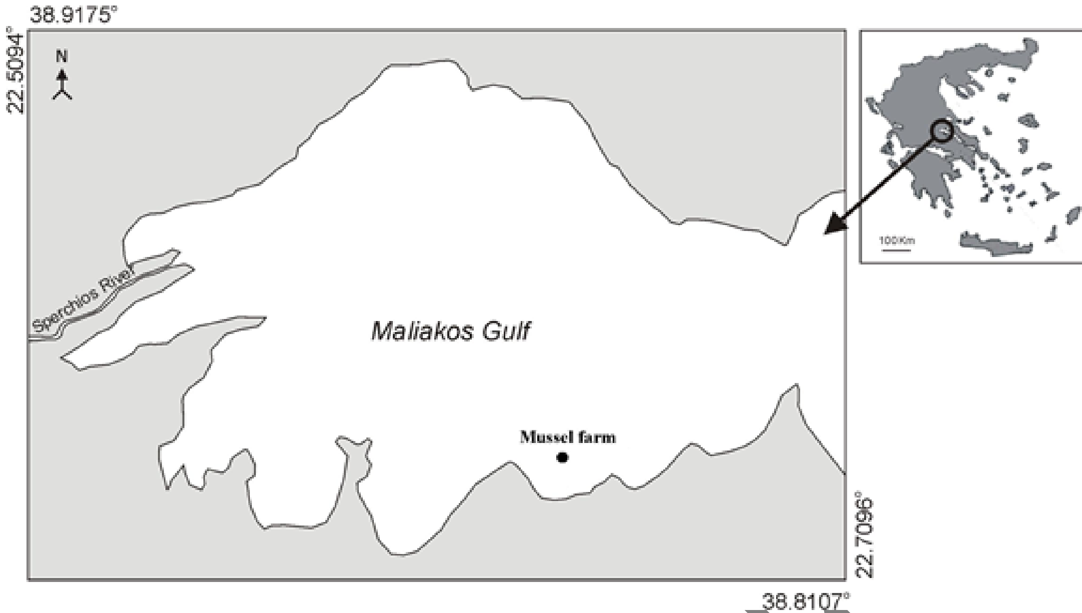

Figure 1. Map of the farm site area in Maliakos Gulf, East Central Greece, (Aegean Sea, NE Mediterranean), where the field *in situ* experimental trials were carried out.

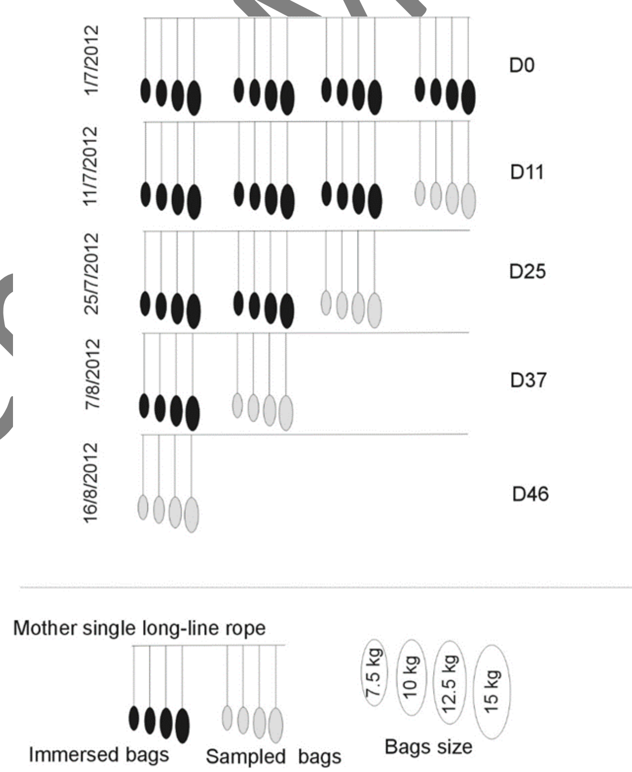

Figure 2 Diagram of mussel bags storage after grading and packing into the single long line floating mussel farm and experimental design.

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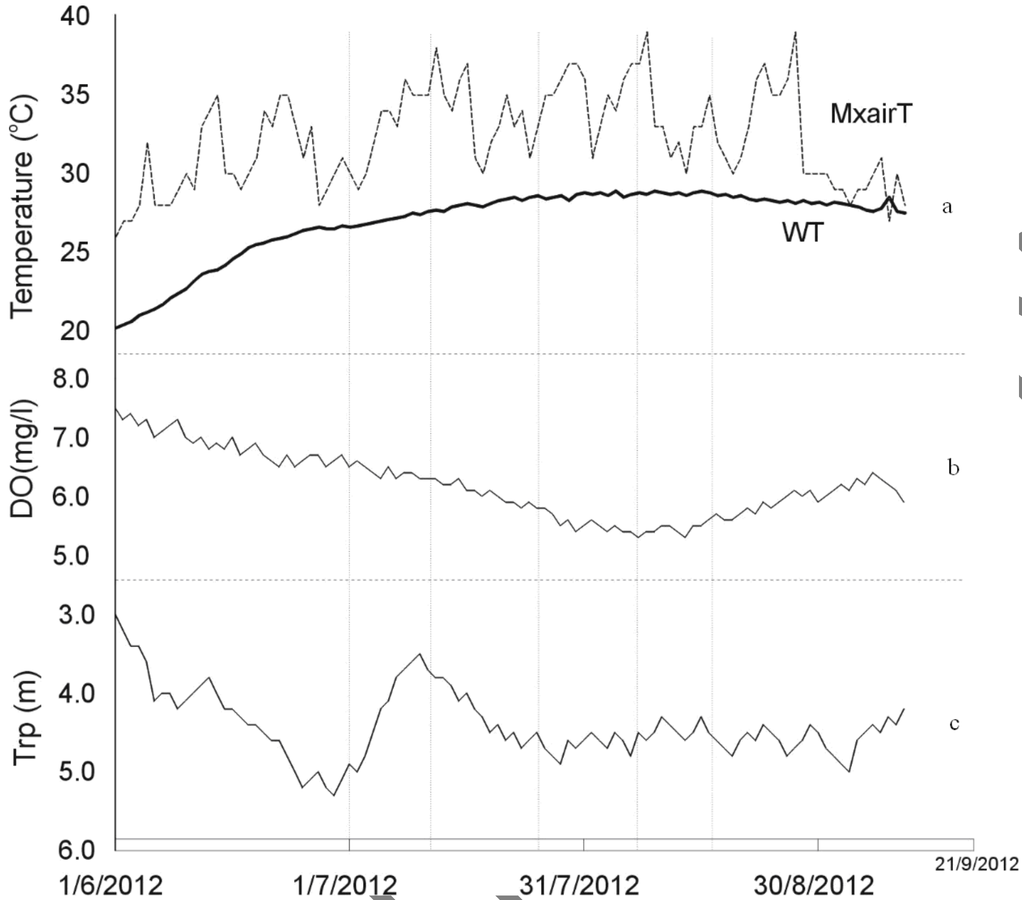

Figure 3. Variation of (a) Water (WT) and Maximum air (MxairT) temperature (°C); (b) dissolved oxygen (DO) levels (mg l^{-1}); and (c) water transparency (Tpr) (m) during summer 2012 (1 June 2012-10 September 2012) in the mussel farm (Maliakos Gulf); Vertical lines mark the date of sampling.

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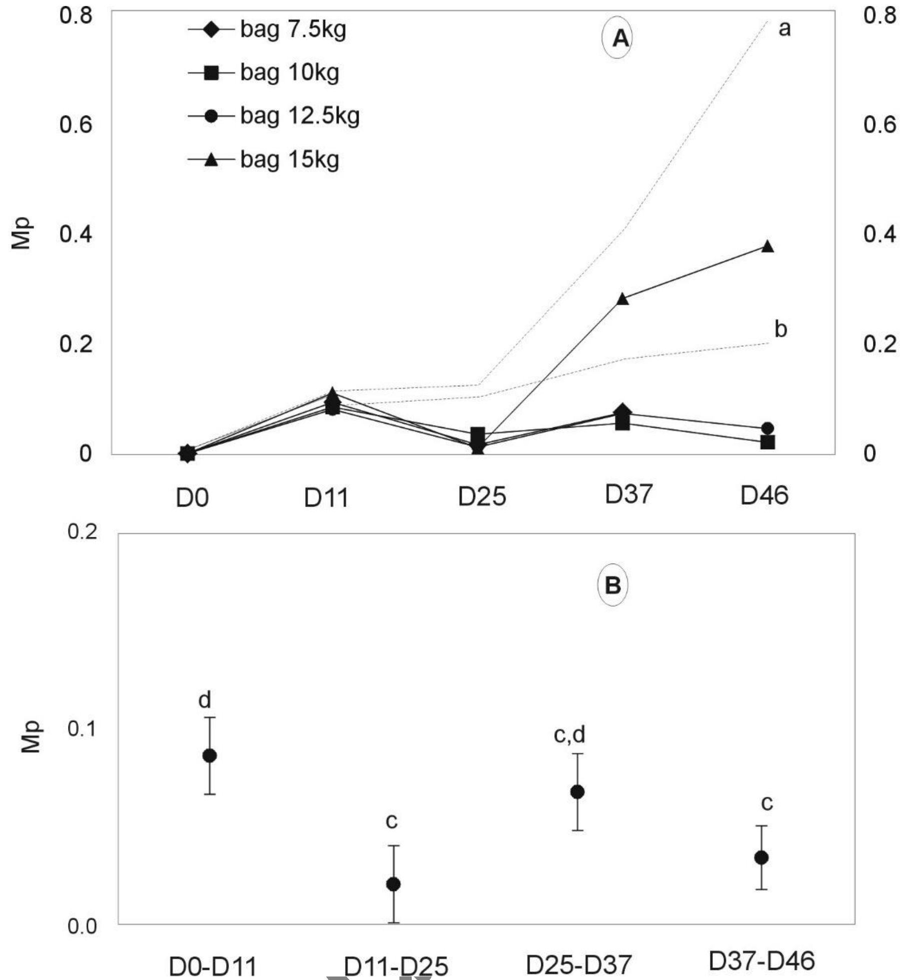

Figure 4. (A) Mussels mortality per time (Mp) bag size and cumulative mortality (Mc), (a) of bag size 15 kg and (b) of others bag sizes, (B) Results of multifactor analysis of variance (Multifactor ANOVA) for mussels Mpfor a 46-day period of re-immersion in summer 2012 (factors: sampling day and bag size; here seems only the statistically significant factor: sampling day). In figure the lower-case letters (c-d), indicating the homogenous groups (Tukey test; P<0.05). The vertical bars correspond on the 95% Confidence limits.