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# Effects of seasonal changes and depth on growth parameters of the Mediterranean mussel (*Mytilus galloprovincialis*) on a shipwreck in the Eastern Mediterranean Sea

by

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## Abstract

The study was conducted between July 2018 and May 2020 to compare seasonal changes in growth parameters of the Mediterranean mussel (*Mytilus galloprovincialis*) at different depths in two different areas of the Northeastern Mediterranean Sea (Aegean Sea). In a preliminary study, mussels were placed in cages at different depths of an artificial reef (AR – shipwreck) and natural reef (NR). Temperature, salinity, pH, and dissolved oxygen were determined seasonally at both sites at a depth of 37 m (deepwater) and 25 m (midwater). Linear increases in length and width of mussels at the AR site were recorded, while no consistent changes were observed for mussels at the NR. There was negative correlation between the specific growth rate (SGR) and salinity ($r^2 = 0.5098$) and temperature ($r^2 = 0.1470$) at the AR site. Negative correlations were also recorded between pH and the commercial condition index (*CCI*) at both the AR ($r^2 = 0.3869$) and NR ($r^2 = 0.3600$) sites. Our findings show that depth had a significant effect ($p < 0.05$) on the *CCI* of mussels at the NR site, while its effect was insignificant for this index at the AR site ($p > 0.05$). This study indicates that natural reefs provide more suitable conditions for the growth of Mediterranean mussels than shipwrecks used as artificial reefs.

**Key words:** Mediterranean mussel, growth, artificial reefs, shipwrecks, Aegean Sea

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# 1. Introduction

The concept of artificial reefs (ARs) is to mimic natural reefs by intentionally submerging structures on the seafloor to serve the aquatic ecosystem by protecting, regenerating, concentrating and/or enhancing living marine resources (Fabi et al. 2015). These man-made structures were deployed to serve as reefs or were made for other primary purposes such as oil platforms, harbors and shipwrecks, and are referred to as secondary artificial reefs (Bortone 2006; Lima et al. 2019). The use of man-made structures underwater to help aquatic environments has probably been continued since the Neolithic period (Ito 2011). Today, ARs have been used in over 50 countries around the world for a number of different purposes (Lindberg & Seaman 2011; Fabi et al. 2015).

In the Mediterranean Sea, artificial reefs have become a highly accepted tool in fisheries management, where they are used to protect coastal areas or sensitive habitats from illegal trawling, and to enhance small scale fisheries (Fabi et al. 2011; Jimenez et al. 2017). Indeed, ARs (e.g. shipwrecks) have been used to create new recreation sites for divers and anglers, and to mitigate the impact of divers on natural reefs in the Mediterranean countries such as Albania, Cyprus, Israel, Malta and Turkey (Borg et al. 2005; Polak & Shashar 2012; Şensurat-Genç et al. 2017). Although there is still a debate on whether ARs should be used or not (Oh et al. 2008), the number of artificial wreck reefs (AWRs) off the Mediterranean coast of Turkey is increasing every day.

Ecological studies on artificial reefs, integrated with biological, biochemical, and material engineering sciences, have been more advanced compared to other scientific fields. Most of these ecological studies (50.6% of the published papers) have been conducted to investigate fish assemblages, as reported by Lima et al. (2019). While most studies have been carried out on vagile animals associated with ARs (Sinis et al. 2000; Lök et al. 2008; Klaoudatos et al. 2012), there are few studies from the Northeastern Mediterranean Sea (Aegean Sea) on sessile animals (Salomidi et al. 2013; Sedano et al. 2019). Although direct and indirect effects of pollutants from existing wrecks on the seabed have been evidenced (ICRAM 2007; Sprovieri et al. 2013), their role on marine ecosystems still attracts little attention, especially in the Mediterranean Sea (Consoli et al. 2015; Sinopoli et al. 2015; Renzi et al. 2017).

The Mediterranean Sea is a biodiversity hot spot, identified by oxygen-rich and nutrient-poor environments (Zenetos et al. 2002; Malak et al. 2011). Shipwrecks and other man-made structures (e.g. artificial reefs) on the seafloor can affect not only nutrient concentrations and oxygen levels by changing current patterns, but can also affect benthic environments, including primacy habitats (Quinn 2006; Ruuskanen et al. 2015; Renzi et al. 2017). New hard substrates in the Mediterranean Sea can be created by artificial structures, thereby causing direct and indirect environmental changes, particularly on sessile animals in their vicinity. The Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819), as a sentinel animal, is commonly used in biomonitoring of environmental pollution due to its ability to accumulate several contaminants (Cajaraville et al. 2000; Goldberg & Bertine 2000; Viarengo et al. 2007; Kayhan et al. 2016; Gedik & Eryaşar 2020). It has the ability to colonize artificial substrates below a depth of 5 m (Fabi et al. 1989; Ardizzone et al. 1996), and to reproduce below 10 m (Renzoni 1963). The growth performance of the Mediterranean mussel is affected by environmental factors such as salinity, food availability, temperature and the amount of nutrients (Bayne & Newell 1983; Lök et al. 2007). With reference to changes caused by shipwrecks, the objective of the present study was to investigate the impact of ARs (shipwrecks) on the growth performance of the Mediterranean mussel by comparing the AR and NR sites off the coast of the Aegean Sea. By comparing the growth performance of Mediterranean mussels at two different locations seasonally, we tested the impact of both reefs on their growth at different depths (25 m and 37 m) during two years. Mussel cages were placed at two different sites in artificial and natural habitats around the Karaburun Peninsula. In this way, the growth performance of mussels on AWRs was investigated and compared for the first time with that in nearby natural (control) sites in the Mediterranean Sea. The main hypothesis of the study was that the growth performance of the Mediterranean mussel would be significantly different between artificial and natural reef sites.

# 2. Materials and methods

## 2.1. Study area

The coastline of the Karaburun Peninsula (KP), with a total length of 130 km, is located in the eastern Aegean Sea. Important economic resources of the region are marine tourism, ecotourism and agriculture. KP is also important in marine aquaculture production with cage farming of sea bream (*Sparus aurata* Linnaeus, 1758) and sea bass (*Dicentrarchus labrax* Linnaeus, 1758). The production of gilthead sea bream, sea bass and Mediterranean mussels is increasing around the peninsula, with an annual production of 15 000 tons, 20 000 tons and 300 tons of shellfish, respectively (İzmir DPAF 2019).

Diving tourism plays an important role in the development of the tourism sector in Karaburun. Therefore, the district governor and diving operators conducted a joint venture to develop this industry. In 2016, two passenger vessels were submerged as artificial wreck reefs (AWRs) on the coasts of Büyükada and Küçükada (Fig. 1). These AWRs are twins (46.6 m total length, 7.9 m beam) and lie at a depth of 36.6 m. One of the wrecks is called "9 Eylül" and lies very close to the natural reef of Küçükada. The other wreck, "Alaybey", is far enough away from the natural reefs of Büyükada, so it was selected as the study site. Designated as a control site, "Aslan Kayası" has the same characteristics and depth as the Alaybey site but has no wreck.

## 2.2. Study design

Mediterranean mussels were obtained from bouncy systems at a fish farm at the beginning of summer 2018. After a two-week acclimation period at the study site, samples were placed into 10 small cylindrical bags (20 samples per bag). To protect the animals from predator attacks, mussel bags were placed in high-density polyethylene cages (50 × 50 × 50 cm) that were covered with PVC-coated nets with a mesh size of 15 mm (Fig. 2A). Mussel cages were deployed at the underwater sites (Fig. 2B), with two cages per site. Cages at both sites were lowered to identical depths of 36 m and 25 m, respectively. At the AWR site, one sample cage was anchored to the seafloor, very close to Alaybey, and the other cage was attached to the main mast of the wreck. At the control site, one cage was on the seafloor, while the other cage was suspended on a rope in midwater, with the same anchoring and bouncy system.

## 2.3. Seawater parameters

Water parameters such as temperature (°C), salinity (PSU), dissolved oxygen concentration (ppm) and pH were measured in situ with a refractometer, an HQD40 oxygen meter, and a WTW pH meter at the time of mussel sampling (between 10:00 and 11:00 a.m.).

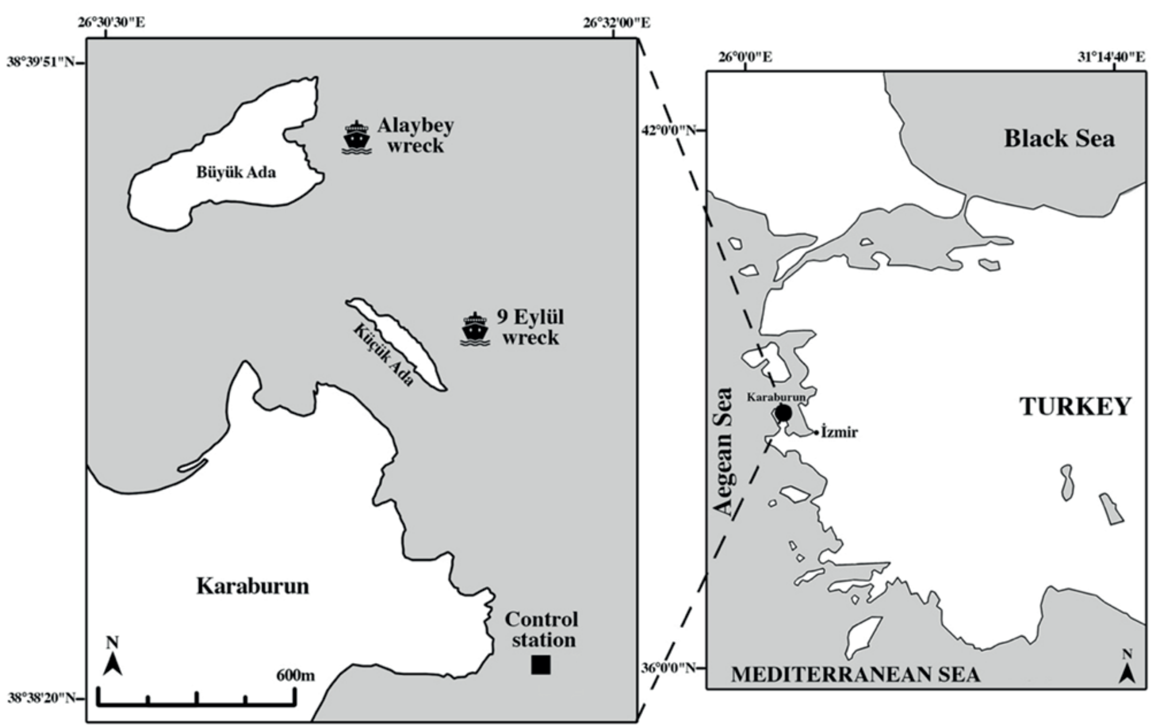


**Figure 1**

Map of the study area. Two passenger vessels (Alaybey and 9 Eylül) are located in the northeastern region of Karaburun, İzmir, Turkey: 9 Eylül extends close to the natural reef of Küçükada, while Alaybey, selected as the study site, is far away from the natural reefs of Büyükada. Aslan Kayası (control site) has similar features to the Alaybey site.

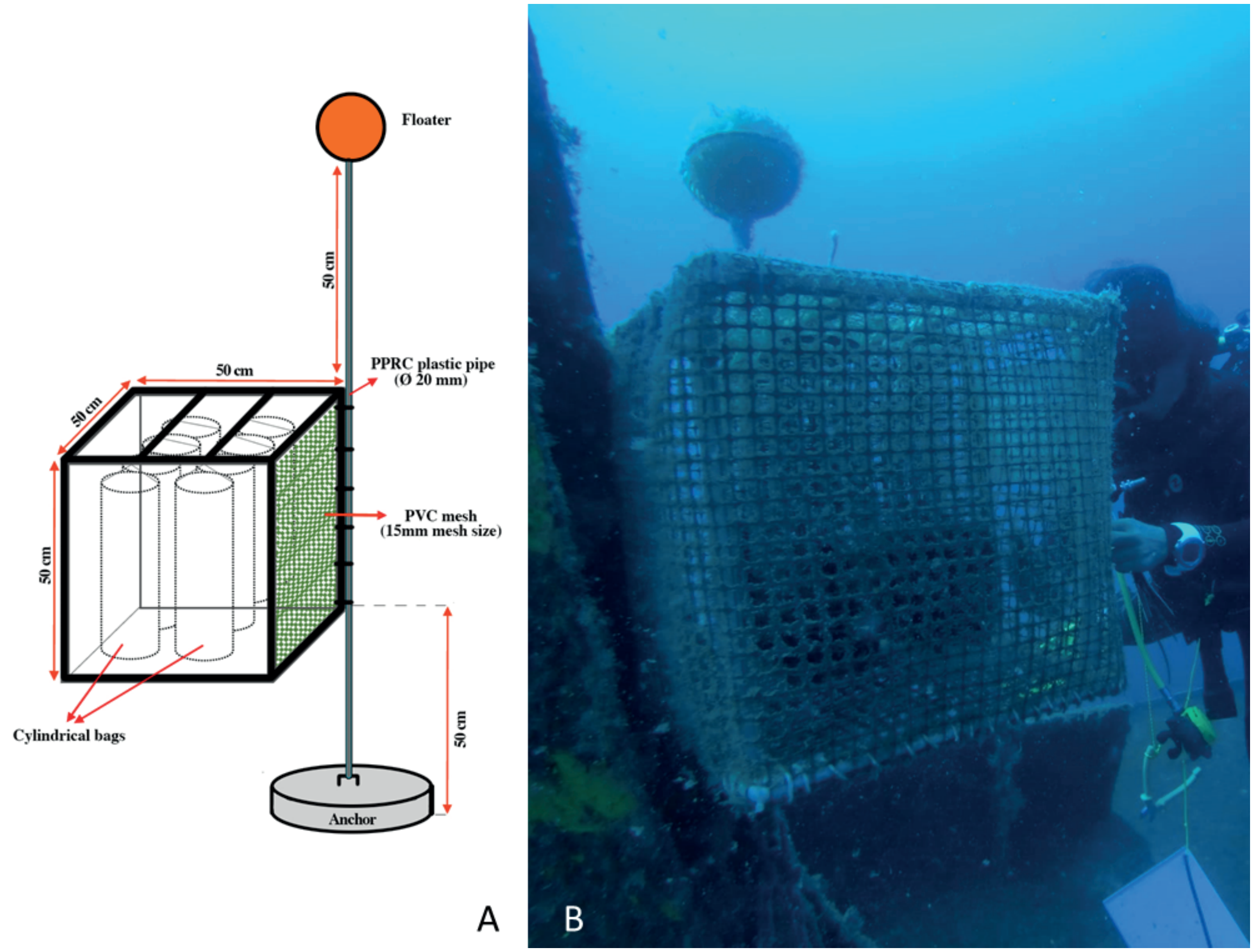


**Figure 2**

Schematic and general view of the mussel cages. A. The mussel cages were deployed under water with an anchor 50 cm below and a float 50 cm above. Mussels were placed into 10 cylindrical bags that were suspended inside the polyethylene cages to protect them from predator attacks. Mussel cages were covered with PVC-coated nets with a mesh size of 15 mm. B. General view of a mussel cage placed at the study site

### 2.4. Sampling and laboratory procedures

For two years, every season between summer 2018 and spring 2020, mussels were retrieved from the cages by scuba divers. The collected samples were stored in cool boxes until they were transferred to the Fisheries Technology Laboratory, Faculty of Fisheries, İzmir Katip Çelebi University. After removing dead animals (open shells) from each sample, the shell length (L, maximum antero-posterior axis) and shell width (W, maximum lateral axis) of the mussels were measured with a digimatic caliper. After the total live weight (TLW) of each animal was weighed using a precision balance (Radwag WTC 200) with an accuracy of 0.001 g, the tissue was dissected from the shell and the total flesh weight (FW) was recorded.

### 2.5. Calculations

The commercial condition index (*CCI*) was calculated according to Hickman & Illingworth (1980) using the following formulae (1):

$$CCI(\%) = \frac{FW}{TLW} \times 100 \quad (1)$$

The specific growth rate in length (SGRL) and the specific growth rate in flesh weight (SGRFW) were also calculated as (2) and (3):

$$SGRL(\%) = \frac{Ln\ Final\ L - Ln\ Initial\ L}{time\ period\ in\ days} \times 100 \quad (2)$$

$$SGRFW(\%) = \frac{Ln\ Final\ FW - Ln\ Initial\ FW}{time\ period\ in\ days} \times 100 \qquad (3)$$

### 2.6. Statistical analysis

Shapiro–Wilk W and Levene's tests were used to check normality and homogeneity of variances, respectively. When normality was rejected, the Kruskal–Wallis test was performed on the annual variation data and the Mann–Whitney U test was used for depth comparisons as well as seasonal and two-year data. Annual interactions between the seasons were ranked with Dunnet's T3 multiple range test. The Pearson correlation coefficient was used to determine the relationship between physicochemical parameters of seawater and SGRL and SGRFW of mussels. All data were processed statistically using Statgraphics Centurion XVI statistical software (Statpoint Technologies Inc., The Plains, VA) (Zar 1999). Differences were considered significant at 5%.

## 3. Results

Seasonal and two-year length (L), width (W), and flesh weight (FW) of Mediterranean mussels placed in artificial and natural reefs are presented in Table 1. Significant differences between AR and NR were found for L ($p = 0.0001$), W ($p = 0.0002$), and FW ($p = 0.0103$) in autumn 2018, and for FW in summer 2019 ($p = 0.0106$) and spring 2020 ($p = 0.0001$). Statistical differences were found for two-year data for all growth parameters, including L ($p = 0.0010$), W ($p = 0.0007$), and FW ($p = 0.0003$). We also calculated two-year SGRFW for both sites. This parameter was estimated as 0.04% and 0.06% for midwater and deepwater AR, respectively. Higher values were also recorded for midwater and deepwater NR, 0.09% and 0.12%, respectively.

The seasonal comparison of annual growth parameters L, W, FW, and *CCI* of Mediterranean mussels at the two different reefs for the first and second year are presented in Table 2. At the AR site in the

**Table 1**

Seasonal (from spring 2018 to spring 2020) and two-year growth parameters of Mediterranean mussels placed in artificial (AR) and natural reefs (NR) in the Aegean Sea

| Period | Reef type | Length (mm) | Width (mm) | Flesh weight (g) |
|---|---|---|---|---|
| Summer '18 | AR | 54.82 ± 2.41 | 27.83 ± 1.12 | 1.94 ± 0.23 |
| | NR | 54.14 ± 3.23 | 27.52 ± 1.52 | 1.99 ± 0.32 |
| | *p* value | 0.8777 | 0.8777 | 1.0000 |
| Autumn '18 | AR | 56.68 ± 0.84 | 29.10 ± 0.43 | 3.69 ± 0.20 |
| | NR | 63.85 ± 1.04 | 32.08 ± 0.50 | 4.65 ± 0.38 |
| | *p* value | 0.0001 | 0.0002 | 0.0103 |
| Winter '19 | AR | 60.94 ± 1.07 | 30.46 ± 0.49 | 4.11 ± 0.26 |
| | NR | 59.60 ± 1.64 | 30.80 ± 1.15 | 3.61 ± 0.34 |
| | *p* value | 0.7125 | 0.9309 | 0.5391 |
| Spring '19 | AR | 61.36 ± 1.46 | 30.83 ± 0.66 | 3.37 ± 0.22 |
| | NR | 65.72 ± 2.71 | 32.72 ± 1.10 | 3.50 ± 0.52 |
| | *p* value | 0.2853 | 0.2287 | 0.7455 |
| Summer '19 | AR | 63.07 ± 1.20 | 31.47 ± 0.58 | 3.28 ± 0.18 |
| | NR | 66.49 ± 1.10 | 32.98 ± 0.73 | 4.09 ± 0.18 |
| | *p* value | 0.0536 | 0.1135 | 0.0106 |
| Autumn '19 | AR | 66.21 ± 1.34 | 32.78 ± 0.61 | 3.00 ± 0.19 |
| | NR | 61.24 ± 2.73 | 31.98 ± 1.21 | 2.84 ± 0.35 |
| | *p* value | 0.1106 | 0.7052 | 0.6101 |
| Winter '20 | AR | 63.30 ± 1.55 | 31.78 ± 0.73 | 4.76 ± 0.51 |
| | NR | 63.06 ± 1.51 | 31.75 ± 0.72 | 4.46 ± 0.40 |
| | *p* value | 0.7260 | 0.9853 | 0.5927 |
| Spring '20 | AR | 64.98 ± 1.08 | 32.71 ± 0.51 | 2.82 ± 0.14 |
| | NR | 67.84 ± 1.45 | 32.41 ± 0.78 | 4.28 ± 0.32 |
| | *p* value | 0.2283 | 0.4912 | 0.0001 |
| Two-year | AR | 61.04 ± 0.47 | 30.79 ± 0.22 | 3.58 ± 0.10 |
| | NR | 64.35 ± 0.67 | 32.33 ± 0.33 | 4.10 ± 0.16 |
| | *p* value | 0.0010 | 0.0007 | 0.0003 |

**Table 2**

Annual growth parameters of Mediterranean mussels placed in artificial (AR) and natural reefs (NR) in the Aegean Sea

| Period | Reef type | Parameter | Summer | Autumn | Winter | Spring |
|---|---|---|---|---|---|---|
| 2018 – 2019 | AR | L (mm) | 54.82 ± 2.41$^{a}$ | 56.68 ± 0.84$^{a}$ | 60.94 ± 1.07$^{b}$ | 61.36 ± 1.46$^{b}$ |
| | | W (mm) | 27.83 ± 1.12$^{a}$ | 29.10 ± 0.43$^{ab}$ | 30.46 ± 0.49$^{b}$ | 30.83 ± 0.66$^{b}$ |
| | | FW (g) | 1.94 ± 0.23$^{a}$ | 3.69 ± 0.20$^{b}$ | 4.11 ± 0.26$^{b}$ | 3.37 ± 0.22$^{b}$ |
| | | *CCI* (%) | 24.11 ± 0.68$^{ab}$ | 23.51 ± 0.08$^{a}$ | 25.00 ± 0.19$^{b}$ | 24.60 ± 0.28$^{b}$ |
| | NR | L (mm) | 54.14 ± 3.23$^{a}$ | 63.85 ± 1.04$^{b}$ | 59.60 ± 1.64$^{ab}$ | 65.72 ± 2.71$^{b}$ |
| | | W (mm) | 27.52 ± 1.52$^{a}$ | 32.08 ± 0.50$^{b}$ | 30.80 ± 1.15$^{ab}$ | 32.72 ± 1.10$^{b}$ |
| | | FW (g) | 1.99 ± 0.32$^{a}$ | 4.65 ± 0.38$^{b}$ | 3.61 ± 0.34$^{b}$ | 3.50 ± 0.52$^{ab}$ |
| | | *CCI* (%) | 24.30 ± 0.66$^{a}$ | 23.81 ± 0.12$^{a}$ | 27.90 ± 0.41$^{b}$ | 27.78 ± 0.38$^{b}$ |
| 2019 – 2020 | AR | L (mm) | 63.07 ± 1.20 | 66.21 ± 1.34 | 63.30 ± 1.55 | 64.98 ± 1.08 |
| | | W (mm) | 31.47 ± 0.58 | 32.78 ± 0.61 | 31.78 ± 0.73 | 32.71 ± 0.51 |
| | | FW (g) | 3.28 ± 0.18$^{a}$ | 3.00 ± 0.19$^{a}$ | 4.76 ± 0.51$^{b}$ | 2.82 ± 0.14$^{a}$ |
| | | *CCI* (%) | 25.56 ± 0.64$^{b}$ | 24.87 ± 0.69$^{ab}$ | 23.09 ± 0.32$^{a}$ | 23.60 ± 0.38$^{a}$ |
| | NR | L (mm) | 66.49 ± 1.10 | 61.24 ± 2.73 | 63.06 ± 1.51 | 67.84 ± 1.45 |
| | | W (mm) | 32.98 ± 0.73 | 31.98 ± 1.21 | 31.75 ± 0.72 | 33.41 ± 0.78 |
| | | FW (g) | 4.09 ± 0.18$^{ab}$ | 2.84 ± 0.35$^{a}$ | 4.46 ± 0.40$^{b}$ | 4.28 ± 0.32$^{b}$ |
| | | *CCI* (%) | 28.50 ± 1.12 | 28.07 ± 1.51 | 28.95 ± 0.42 | 28.50 ± 0.72 |

*L – length, W – width, FW – flesh weight, *CCI* – commercial condition index and different letters in the same line indicate statistically significant differences ($p < 0.05$) among the seasons

first year, the length of mussels in winter and spring 2019 was significantly higher than in summer and autumn 2018 ($p < 0.05$). In winter and spring 2019, the width of Mediterranean mussels was higher than in summer 2018 ($p < 0.05$). The flesh weight of mussels in summer 2018 was the lowest among the seasons ($p < 0.05$), and the meat yield of mussels in autumn 2018 was significantly lower than in winter and spring 2019 ($p < 0.05$). At the NR site, L and W of mussels in autumn 2018 and spring 2019 was higher than in summer 2018 ($p < 0.05$). In summer 2018, the FW of mussels was lower than in autumn 2018 and winter 2019 ($p < 0.05$). The *CCI* of mussels in winter and spring 2019 was higher than in summer and autumn 2018 ($p < 0.05$).

In the second year, no statistical differences were recorded in L and W of mussels placed at the AR site. No differences in L, W, and *CCI* parameters ($p > 0.05$) were found for mussels from the NR site. The highest value of FW was determined for AR mussels in winter 2020, and the *CCI* in winter and spring 2020 was lower than in summer 2019 ($p < 0.05$). For the second period of the experiment, the FW of NR mussels in winter and spring 2020 was statistically higher than in autumn 2019 ($p < 0.05$).

Initial (summer 2018) and final (spring 2020) growth parameters (L, W, FW, and *CCI*) of Mediterranean mussels located in both midwater and deepwater regions of AR and NR are listed in Tables 3 and 4, respectively. Both midwater and deepwater data show significant differences for the FW and *CCI* of mussels in spring 2020 ($p < 0.05$). However, there are also differences between initial and final L and W at both AR and NR for midwater and deepwater ($p < 0.05$). Limit levels of P values were noted for initial and final FW at the AR site for midwater ($p = 0.0547$) and deepwater ($p = 0.0518$) regions. Significant differences were recorded at the NR site for both FW and *CCI* in midwater, but only for FW in deepwater ($p < 0.05$).

Seasonal changes in temperature, salinity, pH, and dissolved oxygen of seawater sampled in the midwater and deepwater regions around the AR and NR sites are shown in Figure 3. In midwater around the artificial reef, the parameters ranged from 14.0 to 28.6°C; from 39 to 44 PSU; from 8.08 to 8.45; from 7.24 to10.79 ppm, respectively. For deepwater, the parameters ranged from 13.9 to 23.1°C; from 39 to 45 PSU; from 8.10 to 8.52; from 7.13 to 10.80 ppm, respectively. In midwater in the vicinity of natural reefs, the parameters ranged from 14.7 to 23.6°C; from 40 to 44 PSU; from 7.96 to 8.61; from 7.36 to 9.48 ppm, respectively. For deepwater, the parameters ranged from 14.5 to 23.2°C; from 39 to 45 PSU; from 8.06 to 8.65; from 7.61 to 10.50 ppm, respectively.

L, W, and FW of Mediterranean mussels in different seasons in the midwater and deepwater regions of the AR and NR sites are shown in Figures 4 and 5, respectively. For midwater data, the length and width parameters of mussels at the NR site were higher than at the AR site for every season, except winters.

**Table 3**

Initial and final growth parameters of Mediterranean mussels placed in midwater of artificial (AR) and natural reefs (NR) in the Aegean Sea

| Period | Reef type | L (mm) | W (mm) | FW (g) | *CCI* (%) |
|---|---|---|---|---|---|
| Summer '18 | AR | 54.75 ± 3.11 | 27.63 ± 1.49 | 1.96 ± 0.25 | 24.36 ± 0.84 |
| | NR | 53.85 ± 3.22 | 27.23 ± 1.57 | 1.95 ± 0.45 | 22.54 ± 0.57 |
| | *p* value | 0.8983 | 0.8983 | 0.7983 | 0.1599 |
| Spring '20 | AR | 65.42 ± 1.50 | 32.78 ± 0.65 | 2.70 ± 0.17 | 23.28 ± 0.41 |
| | NR | 66.71 ± 1.93 | 33.18 ± 0.98 | 3.81 ± 0.37 | 27.15 ± 0.87 |
| | *p* value | 0.7165 | 0.6979 | 0.0127 | 0.0004 |
| *p* value (initial & final AR) | | 0.0099 | 0.0064 | 0.0547 | 0.4755 |
| *p* value (initial & final NR) | | 0.0055 | 0.0089 | 0.0140 | 0.0043 |

*L – length, W – width, FW – flesh weight, *CCI* – commercial condition index

**Table 4**

Initial and final growth parameters of Mediterranean mussels placed in deepwater of artificial (AR) and natural reefs (NR) in the Aegean Sea

| Period | Reef type | L (mm) | W (mm) | FW (g) | *CCI* (%) |
|---|---|---|---|---|---|
| Summer '18 | AR | 54.91 ± 4.09 | 28.06 ± 1.84 | 1.91 ± 0.43 | 22.44 ± 0.64 |
| | NR | 54.49 ± 6.29 | 27.85 ± 2.93 | 2.05 ± 0.48 | 27.32 ± 2.21 |
| | *p* value | 0.9362 | 0.8102 | 0.8102 | 0.0656 |
| Spring '20 | AR | 64.40 ± 1.58 | 32.63 ± 0.85 | 2.99 ± 0.23 | 24.03 ± 0.71 |
| | NR | 69.47 ± 2.21 | 33.74 ± 1.34 | 4.96 ± 0.51 | 30.45 ± 0.95 |
| | *p* value | 0.1196 | 0.6276 | 0.0026 | 0.0003 |
| *p* value (initial & final AR) | | 0.0183 | 0.0314 | 0.0518 | 0.3642 |
| *p* value (initial & final NR) | | 0.0216 | 0.0116 | 0.0056 | 0.2159 |

*L – length, W – width, FW – flesh weight, *CCI* – commercial condition index

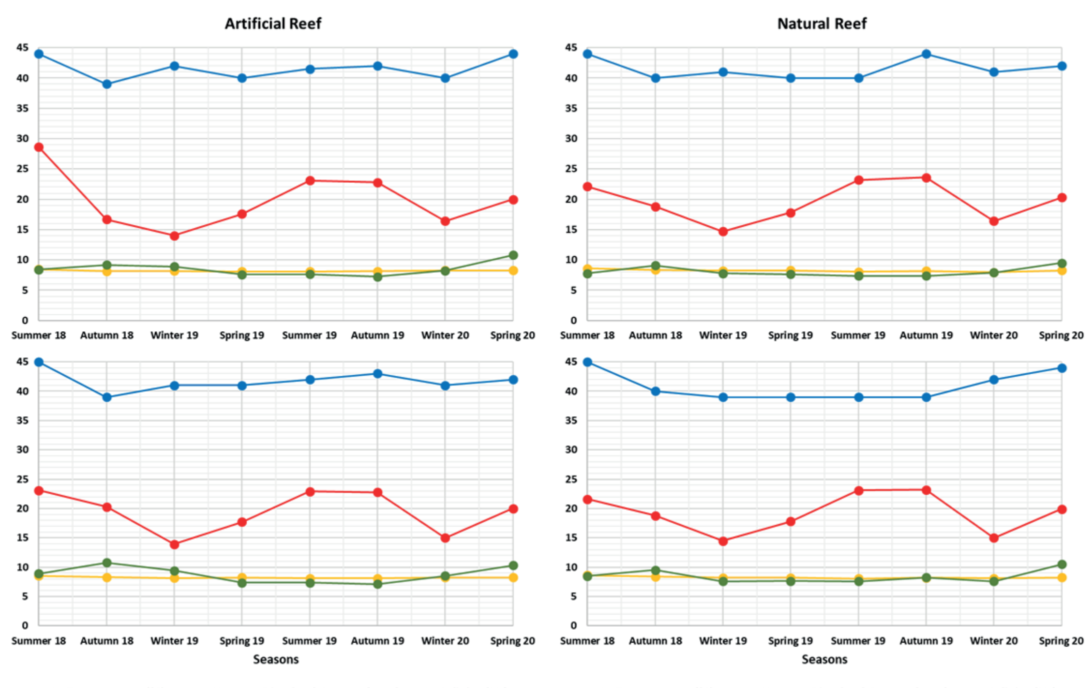


**Figure 3**

Seasonal seawater parameters, including temperature, pH, dissolved oxygen, and salinity of midwater (above) and deepwater (below) regions of artificial and natural reefs

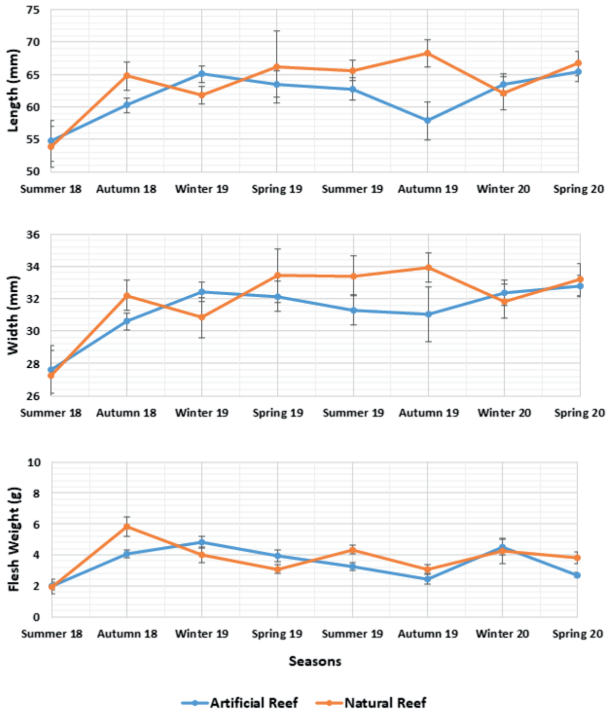


**Figure 4**

Seasonal changes in length, width, and flesh weight (with SE bars) of Mediterranean mussels placed in midwater of artificial and natural reefs

Trendlines with similar breaking points were observed between the L and W parameters (Fig. 4). However, all mussel growth parameters in deepwater cages at the NR site were higher than at the AR site in all seasons (Fig. 5).

Correlations between the physicochemical parameters of seawater and the SGRL and the SGRFW of Mediterranean mussels are detailed in Fig. 6. At the AR site, negative correlations were found between the SGRL and salinity ($r^2$ = 0.5098) and temperature ($r^2$ = 0.1470). Positive correlations were found between the SGRL and DO ($r^2$ = 0.3319), the SGRL and pH ($r^2$ = 0.1706), and the SGRFW and DO ($r^2$ = 0.1044). However, we calculated the correlation between water parameters and *CCI*, and negative correlations were found between pH and *CCI* at both AR ($r^2$ = 0.3869) and NR ($r^2$ = 0.3600). Temperature, dissolved oxygen, and salinity were weakly correlated with *CCI* ($r^2$ ranging from 0.1232 to 0.1989).

## 4. Discussion

Shipwrecks play a certain role in fish diversity as artificial reefs (Consoli et al. 2015; Sinopoli et al. 2015). However, there is no literature that assesses ARWs from this single perspective. Although previous studies have addressed the biodiversity of both fish and benthic species in the vicinity of artificial shipwrecks (Jones & Thomson 1978; Massin et al. 2002; Consoli et al. 2015; Renzi et al. 2017), the effects of abiotic factors on growth parameters of sessile organisms on artificial substrates have not been investigated.

We designed this study to determine and compare the growth parameters of Mediterranean mussels from artificial and natural habitats between summer 2018 and autumn 2020. During these two years, the effects of changing environmental parameters on the growth of mussels was examined seasonally in both areas. At the beginning of the study, even though Mediterranean mussels were observed on guy ropes, masts and hulls of shipwrecks, they were not used as samples to standardize morphometric measurements of mussels. These pre-existing mussels can rapidly colonize artificial habitats (Ardizzone et al. 1989). During this study, we used only caged-reared mussels that survived more than two years. Predator attacks can be considered the main cause of natural mortality of mussels (Seed & Suchanek 1992), thus mussels in this research, unlike in the previous study, were protected by cages.

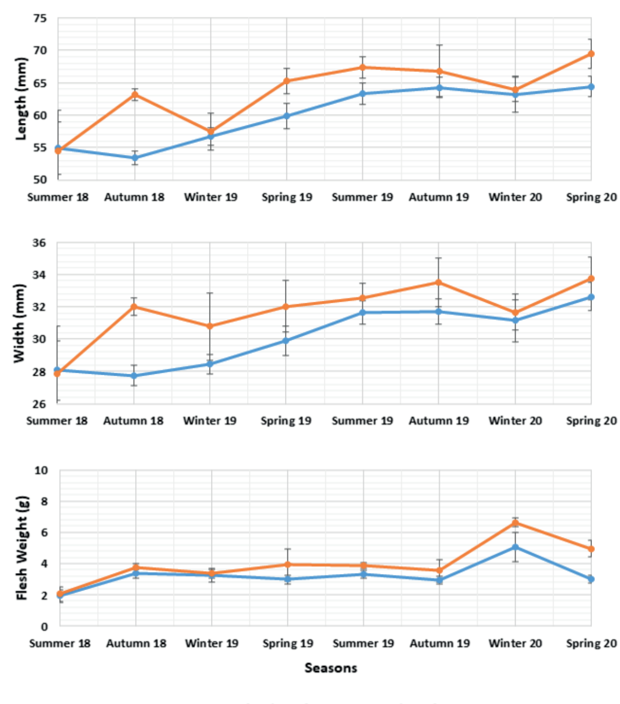


**Figure 5**

Seasonal changes in length, width, and flesh weight (with SE bars) of Mediterranean mussel placed in deepwater of artificial and natural reefs

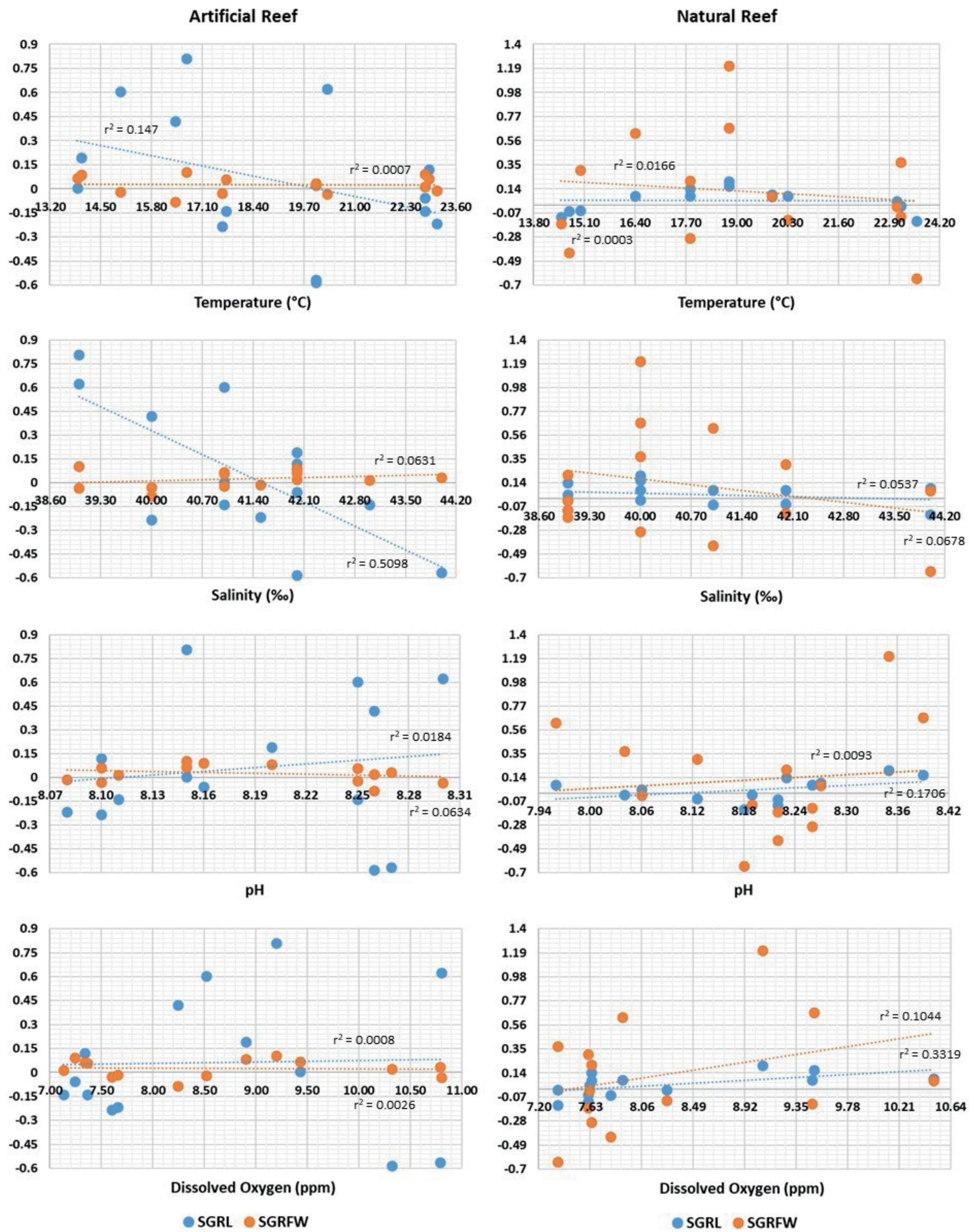


**Figure 6**

Correlations between physicochemical water parameters and the SGRL (%) and SGRFW (%) of Mediterranean mussels placed in artificial and natural reefs in the Aegean Sea (trendlines and $r^2$ values for each growth parameter are shown in the figure)

In this study, biometric measurements (e.g. length and width) were recorded to determine the growth parameters of mussels. At the AR site, increased length and width were observed from summer 2018 to winter 2020. A slight decline was observed in winter 2020, while a recovery phase was observed in spring 2020. Increases in length and width of Mediterranean mussels similar to the AR site were presented in previous studies (Karayücel et al. 2010; Keskin & Ekici 2021). Nevertheless, this pattern did not appear at the NR site. The length and width at the NR site fluctuated with no discernible pattern. Furthermore, seasonal variation in the first year of the study (2018–2019) caused changes in L and W parameters, while no differences were recorded in the second year (2019–2020). Orban et al. (2002) presented fluctuations in biometric parameters of this species in different regions of the Mediterranean Sea. They stated that these parameters are effected by a number of external and internal aspects, including physicochemical characteristics of seawater, the presence of food, and the maturity of mussels. However, seasonal fluctuations were observed in this study for FW and *CCI* of Mediterranean mussels in both midwater and deepwater (at both AR and NR sites). Irisarri et al. (2015) reported that filter-feeding organisms exhibit site-specific differences in quantity and quality of food as a result of natural changes in seston.

Temperature variation is known to be the main factor affecting the development and growth of mollusks (Kapranov et al. 2020). The Mediterranean mussel lives in marine environments where water temperature ranges between 8 and 26°C (Kumlu 2001), and shows optimum growth performance between 17 and 20°C (Blanchette et al. 2007). In this study, temperature values in the midwater and deepwater regions fluctuated during the two years of the study. Mean temperatures of the midwater (19.90 ± 1.67°C) and deepwater (19.46 ± 1.28°C) regions, determined at the AR site during the two years, were at the optimum levels for the Mediterranean mussels. At the AR site, a week but negative correlation was found between the SGRL of mussels and temperature ($r^2 = 0.147$). On the other hand, contrary to some studies (Peharda et al. 2007; Karayücel et al. 2010; Pavičić Hamer et al. 2016), temperature had no impact on the length of mussels at the NR site in this study ($r^2 = 0.0003$). The highest mean FWs were found in winter 2020 at both sites, similar to other studies (Carballal et al. 1998; Orban et al. 2002; Karayücel et al. 2010). Similar to the results of other studies conducted in the Aegean Sea (Lök 2001; Lök et al. 2007; Keskin & Ekici 2021), the growth of mussels at the NR site was slow in winter and increased with increasing temperature from spring to autumn, both in midwater and deepwater. Meanwhile, the same trend was observed in deepwater at the AR site. However, the growth in length decreased in the midwater region of the AR site, despite increasing water temperature from winter to autumn. The structure of artificial wreck reefs can directly affect water currents, and indirectly affect the growth by disrupting food supply (Jimenez et al. 2017). Local currents may provide insight into this unexpected decline in growth in the AR midwater area. This is due to the fact that common currents of the district region were not observed on the seafloor at the AR site, but were observed in all other regions (AR and NR) at 1–2 m above the seafloor (currents felt strongly by divers and the authors' field observations). Local factors can greatly affect the growth rate of bivalves (Peharda et al. 2007; Çelik et al. 2015).

Mean salinity values of midwater and deepwater were 41.56 ± 0.65‰ and 41.75 ± 0.62‰ for the AR site, and 41.50 ± 0.60‰ and 40.88 ± 0.88‰ for the NR site. Although these salinity values represent suitable water conditions for Mediterranean mussels (Kumlu 2001), lower salinity values (ca. 35 PSU) were reported by some authors in different regions of the Mediterranean Sea (Parisi et al. 2005; Keskin & Ekici 2021). There are several studies that assessed the effects of salinity on physiological performance of the Mediterranean mussel (Shurova 2001; Hamer et al. 2008). Despite the wide salinity tolerance of *Mytilus edulis*, *M. galloprovincialis* can be affected by low salinity. Therefore, the Mediterranean mussel is cultured at higher salinity (< 34 PSU; FAO 2017). Salinity values in our study were higher than the optimum levels for mussel cultivation. Based on our findings, salinity shows a negative correlation with the SGRL of mussels at the AR site ($r^2 = 0.5098$). Similar findings were reported at shallow waters of the Aegean Sea (Yıldız et al. 2013). Changes in seawater salinity cause changes in metabolic, energetic and oxidative condition of Mediterranean mussels. Freitas et al. (2017) observed that these changes can alter the physiological status of organisms such as growth performance and reproductivity. This relates to species survival in a changing world.

The pH remained at optimum levels for the growth of the Mediterranean mussel during the study period at both AR and NR sites (8.21 to 8.27). Similar to our results, Bamber (1990) and Michaelidis et al. (2005) reported that shell length of *M. galloprovincialis* and *M. edulis*, respectively, were correlated with pH. While those studies showed a positive correlation, the present study found a negative correlation between pH and L. The response of marine bivalves to a decrease in pH in the seas caused shell dissolution and growth inhibition, and even resulted in mortality. According to Michaelidis et al. (2005), marine bivalves at pH of 7.3 show a 55% reduction in their growth. The same author also indicated that marine bivalves cannot tolerate pH ≤ 7.0. Moreover, other researchers showed that mortality of *M. edulis* increased, while the condition index decreased as pH dropped (Sun et al. 2016). In the present study, a negative correlation was found between pH and the commercial condition index at both the AR ($r^2 = 0.3869$) and NR ($r^2 = 0.3600$) sites. Mussel condition indices show seasonal variation (Okumuş 1993; Gvozdenović et al. 2020). For both mussels from the AR and NR sites, the *CCI* changed. It depended on the season, in the first year of the study, with an increase in winter at both sites. Similar results for *CCI* were reported by Yıldız et al. (2006) in the Dardanelles. The opposite results were observed for environmental parameters in spring and summer. They were found more favorable for the growth of mussels than in winter (Garen et al. 2004; Rouane-Hacene et al. 2015; Kovačić et al. 2017). However, there is no correlation between temperature and *CCI* of mussels for both sites, as reported in previous studies (Karayücel et al. 2010; Irisarri et al. 2015).

In addition to temperature variability, the level of oxygenation is one of the important factors affecting the cultivation of bivalves (Prins et al. 1995; Kapranov et al. 2020). The mean dissolved oxygen concentrations in midwater and deepwater were 8.50 ± 0.40 ppm and 8.73 ± 0.50 ppm at the AR site, and 8.05 ± 0.28 ppm and 8.41 ± 0.38 ppm at the NR site. In the second cold period of the study, i.e. from autumn 2019 to spring 2020, dissolved oxygen concentrations increased in both regions of the AR site, similar to what was reported by Kapranov et al. (2020). In the last season of the study, i.e. spring 2020, the highest dissolved oxygen concentrations were measured at all mussel cage layers, possibly due to a phytoplankton bloom. According to experimental studies on the effects of depth, even though the oxygen concentration decreases with depth, mussels can adapt to different depths, that is, their gills can function (Galgani et al. 2005). In spring 2020, when it was assumed that nutrients were available, the presence of the shipwreck may have affected the growth performance of the mussels. While there were no differences between the sites in the growth of mussels (both L and W), the mean length of mussels at the NR site was larger than that at the AR. A positive correlation was also found between dissolved oxygen and the SGRL at the NR site ($r^2 = 0.3319$). This can be explained by the fact that there may be no correlation between depth and growth performance when temperature and light are not limiting the presence of nutrients. Galgani et al. (2005) reported no statistical differences at $P < 0.01$ related to depth in the condition index (dry weight/max shell length). In our findings, there are statistical differences between FW and *CCI* of mussels at both AR and NR sites for both midwater and deepwater levels (Table 3). In addition to differences between the sites, the *CCI* of mussels differed at different depths (25 m and 37 m) at the NR site ($p < 0.05$). Although the effect of depth on the *CCI* of mussels was insignificant at the AR site ($p > 0.05$), the *CCI* values from deeper cages were higher than those from midwater cages.

The structure of artificial reefs can play an important role in the process of larval settlement, adult settlement (Perkol-Finkel & Benayahu 2007) and food supply for fouling organisms such as bivalves (Walker et al. 2007). These structures can, in fact, enable faster growth (Jimenez et al. 2017). AWRs increase habitat complexity and offer a number of shelters and hard bottom surfaces for juvenile and adult animals (Zintzen et al. 2006). However, some environmental changes (e.g. less light, local disturbing currents and pollutants) created by artificial reefs (shipwrecks) may create conditions that are not ideal. In our study, no positive effect of the artificial reef on the growth of Mediterranean mussels was found compared to the natural reef. Consequently, we should note that our understanding of artificial reefs (shipwrecks) is limited when considered from only one perspective. Each shipwreck exhibits specific characteristics for the marine environment in terms of chemistry, biology, ecology, health, and even economy (diving, fishing). Therefore, shipwrecks should be researched from a broad perspective to determine their potential impacts or risks on animals and even humans.

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