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Effect of Fish Farming on Bioecological Characteristics of the Mediterranean Mussels *Mytilus galloprovincialis* Lamarck, 1819 (Bivalvia: Mytilidae): Güllük Bay Example, South Aegean Sea, Turkey

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Abstract: This study was carried out to determine the effects of cage cultivation on bioecological characteristics of *Mytilus galloprovincialis* in Güllük Bay Kazıklı Port between May 2013 and May 2014. An integrated multi-trophic aquaculture method was used. The growth and survival rates, age structure and reproductive success of mussels as well as water quality parameters and phytoplankton composition were monitored monthly in two selected stations. Mussels belonging to different size groups exhibited high growth and survival rates during the summer and spring when the temperature was high near the offshore systems. Applying a length-frequency analysis graph created using the shell growth limits, it was found that the mussels reached the marketing length (>50 mm) in 18 months. With this study, it has been revealed that mussel development continued in both stations and the region was suitable for potential mussel farming.

Key words: Aquaculture, growth, reproduction, phytoplankton composition

Introduction

Aquaculture constitutes an important industry in Güllük Bay, South Aegean Sea, Turkey. Using offshore aquaculture systems has been an ongoing activity for many years in this area. Although the fish production and the consumption potential are high, the tradition of mussel farming in the region has been limited until recently. Despite mussels have been the best-known type of shellfish in the area, aquaculture studies have been deficient. In recent years, the Aegean Sea coast of Turkey, like many other coastal areas in the Mediterranean, has been developing rapidly in terms of meeting the socioeconomic interests of the country (Sunlu 2006). Mussel farming is becoming widespread along the Turkish coast and the production is gradually increasing (Serdar et al. 2019).

The communities of the Mediterranean mussels *Mytilus galloprovincialis* Lamarck, 1819 along the Mediterranean coastal areas are essential bioindicators as they can grow in clean or semi-polluted regions and are sensitive to chemical pollutants (Damianidis & Chintiroglou 1998). In mussel culti-

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vation, the growth rate and gonadal development are mostly dependent on environmental factors such as population density and salinity as well as on food availability and temperature parameters (Sastry 1975, Bayne & Newell 1983). There are many studies on reproductive cycle and cultivation of *M. galloprovincialis* in different regions throughout the world (e.g., Villalba 1995, Curiel-Ramirez & Caceres-Martinez 2004, Garen et al. 2004, Sarà et al. 2009, Okaniwa et al. 2010, Irisarri et al. 2015, Keskin et al. 2020). Several studies have been done on the heavy metal contents of this species in Güllük Bay (Sunlu 2006, Kucuksezgin et al. 2013, Belivermiş et al. 2016).

The aim of the present was to determine the effect of mussel farming on the water quality in Güllük Bay, an area where fish farming is carried out. In addition, by determining the growth, survival, nutritional and reproductive performance of the mussels, we also aimed to evaluate the potential capacity and sustainable use rates for aquaculture production in the region.

Materials and Methods

Experimental design

Two stations were established in Güllük Bay, Kazıklı Port:

(1) Station I, coordinates 37˚15′18″N, 27˚26′93″E, total depth 51 m, was an offshore aquaculture area used for producing sea bass and sea bream.

(2) Station II, coordinates 37˚15′18″N, 27˚26′93″E, total depth 8 m, was located near the coast, away from the offshore aquaculture system.

The study was designed in two periods. The first period was May 2013 – October 2013. The second period covered November 2013 – May 2014. In both periods, mussels were collected from nature and divided into size groups. The study lasted 366 days.

In the study area, 480 mussels were collected at once before the start of each period. All the mussels were collected over the ropes of the mooring system used around the net cages. They were divided into two size groups: medium-size group (MS) and large-size group (LS). In the first period, the medium-size group was divided into the Station I (initial mussels' length av. 42.95 mm) and the Station II (initial length av. 40.08 mm). The large-size group had an initial length of av. 64.52 mm in the Station I and av. 65.89 mm in the Station II. In the second period, the medium-size group was divided as follows: av. 37.75 mm length for the Station I and av. 35.14 mm length for the Station II. The large-size (LS) group was av. 56.82 mm for the Station I and av. 60.37 mm length for the Station II.

The collected mussels were used for each period of study. The net breeding model was applied. To avoid the mussels touching the bottom, they were placed at a depth of 12 m in the Station I and of 5 m in the Station II.

Mussel growth, survival performance and age

Throughout the study, shell length (SL), shell width (SW) and shell height (SH) were measured monthly with a digital calliper (0.02 mm accuracy). The total weight (TW) was measured with an electronic balance (0.01 g accuracy). The growth of the mussels was calculated based on their weights and lengths through the equations given below (Chatterji et al. 1984, Malouf & Bricelj 1989, Gaygusuz et al. 2013):

$$\text{Instantaneous growth rate (IGR)} = (\ln S2 - \ln S1)/(t2-t1),$$

where S1 and S2 were respective size measurements (length, L; weight, W) at the beginning and end of the study, respectively. The duration of the study (in months) was expressed by t, (t2-t1).

$$\text{Relative growth rate (RGR \%)} = (W2-W1/W1) \times 100,$$

where W2 and W1 were the final and initial weight, respectively.

$$\text{Specific growth rate (SGR)} = [(\ln W2 - \ln W1)/t] \times 100,$$

where W1 was the individual mean initial weight, W2 was the individual mean final weight and t was the duration of the study period in days.

$$\text{Survival rate (\%)} = Ntx100/N0,$$

where Nt was the number of live mussels after time t and N0 was the number of mussels at the beginning of the experiment.

Age estimations were done using the shell growth limits and length-frequency analyses of mussels (Polat et al. 2008).

Reproductive performance

Mussels' sex and maturity stages were determined by microscopic examination of male and female gonads. Mussels used for histological analysis were fixed by sectioning the adductor muscle and intro-

ducing it to neutral buffered formalin (10% concentration) for at least 24 h. Gonad taken from each mussel was passed through the fixation sequence as described by Hillman (1993). In the fixation process, steps such as dehydration, clearing and infiltration were applied. After paraffin embedding in histological cassettes, 3–5 µm sections were taken from the gonad tissues using a Leica 295 microtome. Then, gonad tissues were stained with haematoxylin and eosin. Each slide was examined using an Olympus CX31 microscope to determine the sex and gonadal development stage. The photomicrograph of the male and female gonads was taken with a Leica DFC 290 digital camera. The nomenclature of the gonad development stages in *M. galloprovincialis* followed that used in previous studies (Seed 1976, Hillman 1993, Gray et al. 1997, Barber et al. 2005, Suarez et al. 2005, Lök et al. 2011). If ovarian and testicular tissues were observed in the same gonad, the mussel was classified as a hermaphrodite.

Environmental parameters

Physical and chemical parameters (temperature, oxygen, pH, electrical conductivity and salinity) were measured monthly at the two stations using the YSI Professional Water Quality Meter Plus Quatro-20m. Visibility and colour of seawater were measured *in situ* using a Secchi disc and Forel scale. Samples that could not be analysed within one hour were put into 2L polyethylene sampling bottles and analysed in the laboratory without breaking the cold chain (APHA 2000). Spectrophotometric analyses in seawater samples were performed using methods 4500 NO_3-E for nitrate nitrogen, 4500 NO_2-B for nitrite nitrogen and 4500 NH_4-F for ammonium nitrogen. For eutrophication monitoring and evaluation, nutrient salts, oxygen, chlorophyll-a concentrations and TRIX index values were calculated using the following equation (Vollenweider et al. 1998):

$$TRIX = (Log10\,[ChA \times aDO\% \times DIN \times TP] + k) / m,$$

where ChA = chlorophyll-a concentration, aDO% = Oxygen as absolute, DIN = dissolved inorganic nitrogen, DIN = N(as $N\text{-}NO_3$+$N\text{-}NO_2$+$N\text{-}NH_4$), TP = total phosphorus. The parameters k =1.5 and m =12/10 = 1.2 are scale coefficients introduced to fix the lower limit value of the index and the extension of the related trophic scale from 0 to 10 TRIX units.

Analysis of variance (Two-way ANOVA) was applied to determine the effects of seasonal and station factors on the changes in environmental parameters obtained from two stations. The statistical difference between the two periods was used to determine the seasonal effect.

Phytoplankton composition

Vertical sampling was carried out with a plankton mesh with a 20-µm mesh to determine phytoplankton composition at the stations. The precipitation method was applied by adding 4% formaldehyde to the samples. Counts were started after homogenization was achieved. In the counting process, direct counting was made using the Neubauer counting chamber and the amount of cells per litre was calculated (Utermohl 1958). Species were identified with a Nikon E200 brand-model microscope. The taxonomic determination of the samples was based on Wood (1968), Cupp (1977), Balech (1988) and Tomas (1997).

Results

Environmental and water quality parameters are presented in Fig. 1. The Trophic Index values were less than 3 and, therefore, this aquatic environment was oligotrophic. The distributions and densities (Fig. 2) of phytoplankton organisms were showed variations in the two sampling stations. In the first station, located in the region of the net cages where fish farming was carried out, the most abundant taxon was *Gyrodinium* sp. identified in autumn and spring. In the second station, *Prorocentrum* sp. was detected at a high rate during the sampling year.

The average shell lengths and weights of mussels (Fig. 3) demonstrated instantaneous growth, both relative and specific growth rates (SGR) by height and weight (Table 2). The highest instantaneous growth by height and weight in both stations was in mussels belonging to the 1st size group. It was determined that the lowest instantaneous growth was in mussels belonging to the 2nd size group in the 2nd period at both stations. Mussels belonging to the medium-size group were found to develop faster in height and weight than those in the large size group.

The length-frequency analysis using the survival rates and shell growth limits of the mussels at both stations is given in Fig. 4. The mussel lengths collected around each peak are assumed to have a normal distribution according to the Shapiro-Wilk test, and each peak corresponds to a different age group. The first peak (30 mm) of the smallest individuals constitutes the first age class. The second-age group consists of mussels with a length of 30–60 mm. Mussels of the size-groups above 60 mm are considered over two-years old.

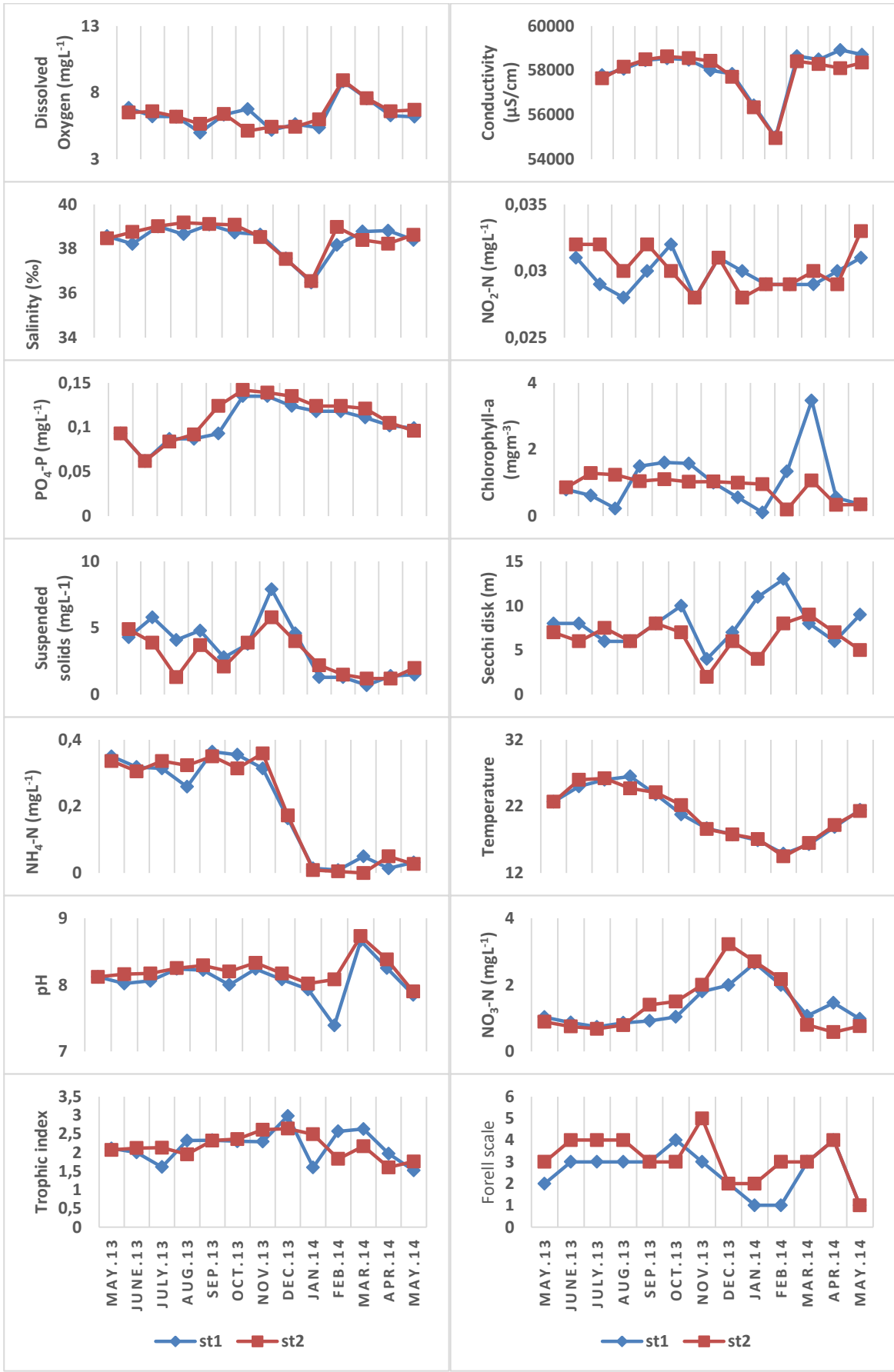

Fig. 1. Monthly environmental variables at sampling stations in Güllük Bay

Table 1. Mean values with ANOVA for water quality parameters in sampling area

	Station I		Station II	
	Period I	**Period II**	**Period I**	**Period II**
Avg. Temperature (0C)	24.8 [a]	18.2[b]	24.7[a]	18[b]
Avg. pH	8.1[a]	8.1[a]	8.2[a]	8.4[a]
Avg. Dissolved Oxygen (mgL^{-1})	6.1[a]	6.5[a]	6.3[a]	6.4[a]
Avg. Conductivity (µS/cm)	58264[a]	57754[a]	58295[a]	57458[a]
Avg. Salinity (‰)	38.7[a]	38.2[a]	38.9[a]	38.2[a]
Avg. NO_3-N (mgL^{-1})	0.88[a]	1.62[b]	0.9[a]	1.85[a]
Avg. NO_2-N (mgL^{-1})	0.03[a]	0.03[b]	0.031[a]	0.029[b]
Avg. NH_4-N (mgL^{-1})	0.321[a]	0.119[b]	0.33[a]	0.13[b]
Avg. PO_4-P (mgL^{-1})	0.084[a]	0.118[a]	0.091[a]	0.127[b]
Avg. Chlorophyll-a (mgm^{-3})	0.952[a]	1.121[a]	1.11[a]	0.806[a]
Avg. Suspended solids (mgL^{-1})	4.4[a]	2.8[a]	3.2[a]	2.8[a]
Avg. Secchi disk (m)	7.2[a]	8.5[a]	6.9[a]	6.1[a]
Avg. Trophic index	2[a]	2[a]	2[a]	2[a]
Avg. Forell scale	3[a]	2[a]	4[a]	3[a]

* The different letters indicate statistical difference among zones at $p<0.05$)

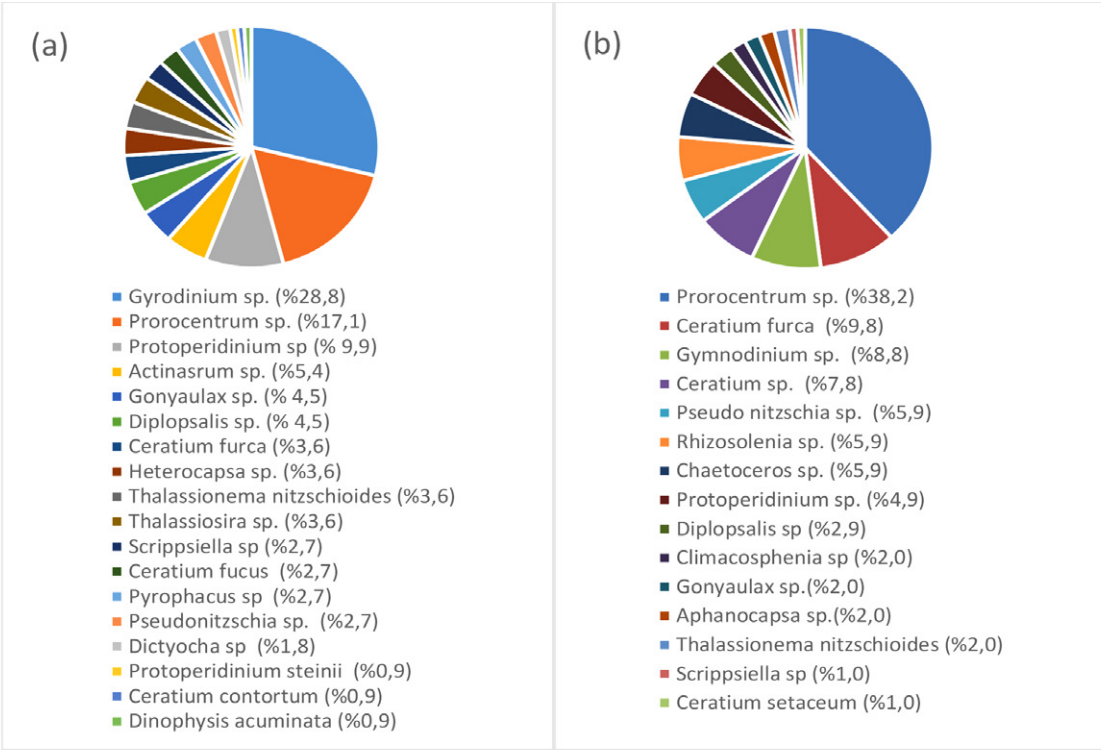

Fig. 2. Phytoplankton densities in Station 1 (a) and Station II (b)

Samples of gonads of specimens with identified gender affiliation were studied. All the six developmental phases were examined: rest, development, maturity, fertilization (ovulation), restoration and degeneration phases were detected (Fig. 5). During the resting phase (Phase I), sperm and eggs were not distinct and the gonad were covered with connective tissue. Both male and female individuals were found in the resting phase in April, May, June, July, August, September and October.

During the development phase (Phase II), which is characterised with early stages of spermat-

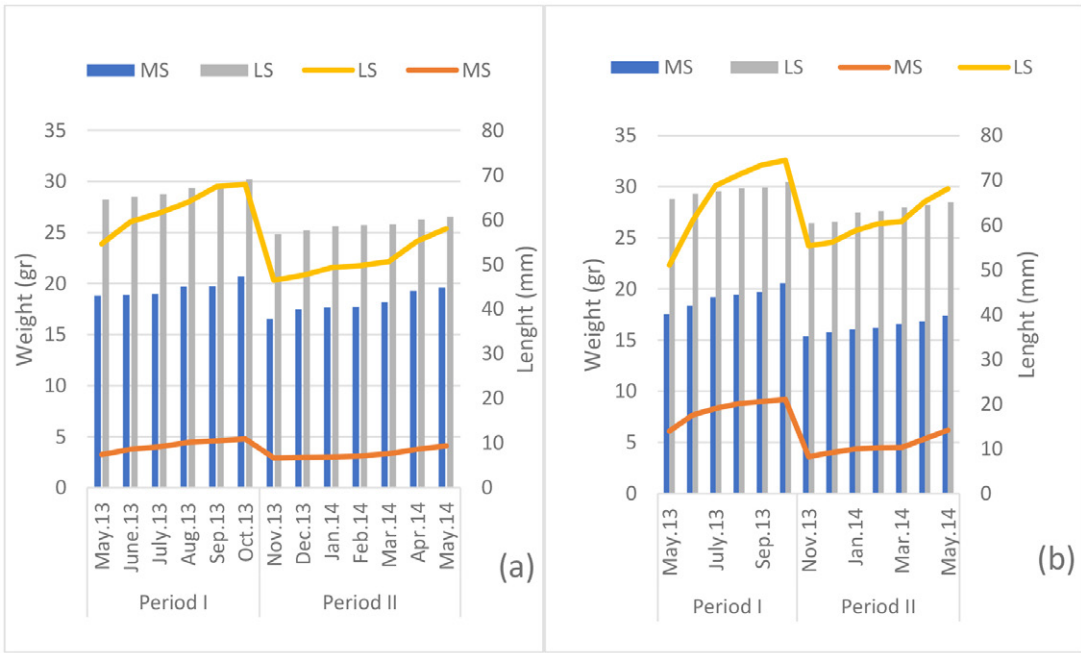

Fig. 3. Monthly growth in Station I (a) and Station II (b) of mussels.

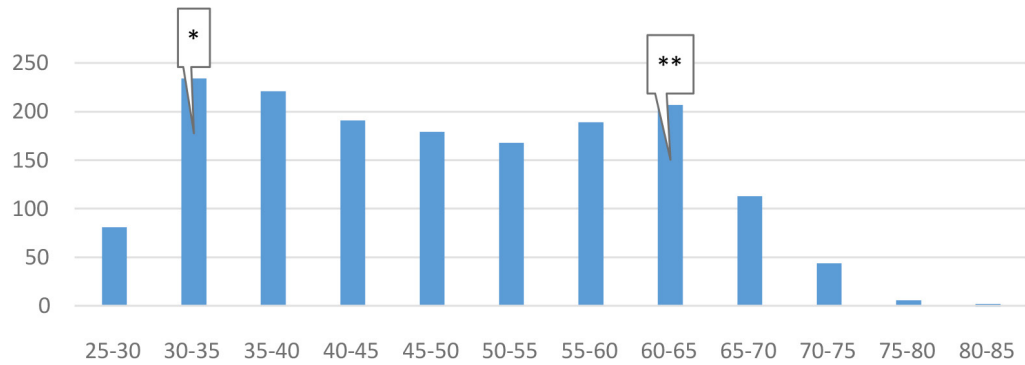

Fig. 4. Length–Frequency Analysis for age (n=720, *and**: Possible ages)

ogenesis or oogenesis, follicles began to form in the connective tissue in male and female individuals. In these follicles, oocytes were seen in females and spermatogonia were distinct in males. During the study period, the development phase in female individuals was detected in May, June, July, September and October, while, in males, it phase was detected in May, June, and August. Individuals ready for fertilization in the maturity phase (Phase III), which was characterised by the presence of follicles filled with germ cells, were also present; in them, the connective tissue between cells is completely reduced. Mature oocytes were seen in females. At this stage, in males, the follicle is swollen and combined, and most were filled with spermatozoa. In this study, we determined that male and female individuals were in maturity between May and March.

Gaps are seen in the follicles during the fertilization phase (Phase IV). In female individuals, egg expending from gonads occurs. The number of mature oocytes in the gonad canal was decreasing. In male individuals, a sperm throw was performed. In this study, the ovulation phase in male and female individuals was observed between December and May.

Successive restoration of sex cells is seen in individuals in the restoration phase (Phase V). In this study, mature oocytes began to appear in follicle lumens in females during the restoration phase. In males, spermatocytes and spermatids began to increase at this stage. Fertilization occurred again with this rapid restoration. The restoration phase was determined in September, November and March in females while, in males, it was found in October only.

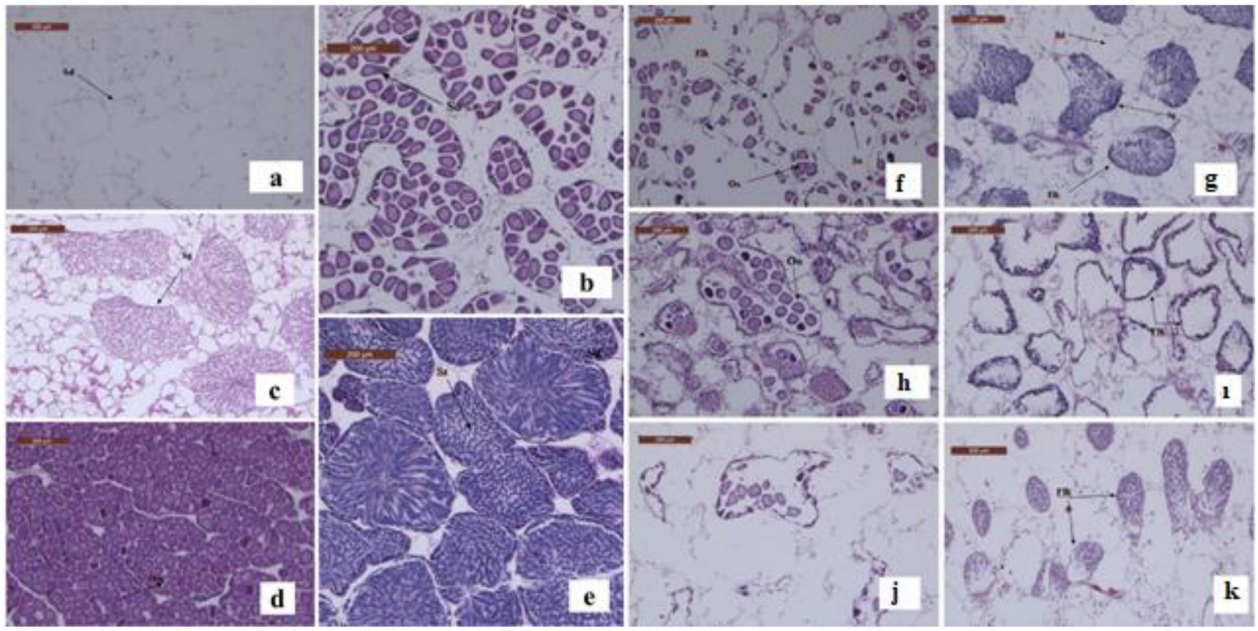

Fig. 5. a) Gonad section in the resting stage (bd: connective tissue), b) Development stage in the female individual (So: Oocytes with stalks), c) Development stage in the male individual (Sg: Spermatogonia), d) Gonad section in the mature female individual (Oo: Mature oocyte), e) Maturity stage male individual gonad section (Sz:spermatazoa, Sg: Spermatagonia), f) IV. stage (insemination) female individual (Flk: Follicle, Oo: Mature oocyte, So: Stalked oocyte), g) IV. stage (discretion) male individual, h) V. stage (gamete regeneration) female individual ı) V. stage male individual, j) VI. Stage (deterioration) female individual, k) VI. Stage male individual

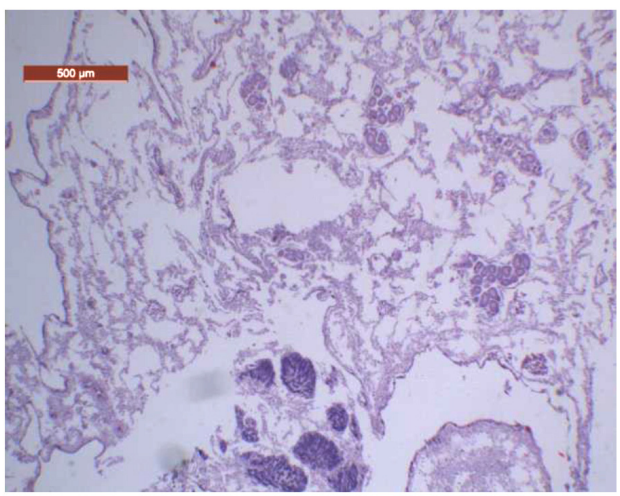

Fig. 6. Hermaphroditic gonad section

During the degeneration phase (Phase VI), the follicles were empty or destroyed. The stage of deterioration in female individuals was between May and October, while, in male individuals, it was in April, June and September.

In some cases, hermaphrodite mussels were observed. Such an individual was identified in the sample from the station 2 in April (Fig. 6).

Discussion

It is believed that the most important factor affecting growth of mussels is the temperature (Jones & Iwama 1991). According to Min (2011), the growth of mussels slows down during the long and cold winter seasons. The adaptation not only of cultivated mussels but also of some economically important fish species cultivated in the Aegean Sea demonstrated that the temperature regime is the leading limitation factor in the region.

It is accepted that the standard shellfish aquaculture waters are characterised by salinity values equal to or less than 40‰. Although it is known that marine mussels can survive at the lowest salinity rate of 4-5‰, they are not effective when the salinity value is gradually reduced (Kautsky et al. 1990). In our study, it was found that the Güllük Bay Kazıklı Port had salinity values suitable for mussel cultivation.

In September and November, when the highest ammonium nitrogen was detected, there was a decrease in phytoplankton biomass at the stations. Between November and January, there was an increase of the fish biomass in the production system. Ammonium, which has not been used by phyto-

Table 2. Differences in the number of individuals, survival rates, size measurements (weight, length), instantaneous growth rate, specific growth rates and relative growth rate as percentage of *Mytilus galloprovincialis* at the beginning and the end of the study from two sampling stations in the Güllük Bay. MS (Medium Size), LS (Large Size)

Characteristics	Station 1				Station 2			
	1st Period		2nd Period		1st Period		2nd Period	
	MS	LS	MS	LS	MS	LS	MS	LS
Initial no. of individuals	120	120	120	120	120	120	120	120
Final no. of individuals	87	94	84	81	85	77	95	82
Survival rate (%)	73	78	70	68	71	64	79	68
Initial length (mm)	42.95 (±1.8)	64.52 (±0.8)	37.75 (±1.9)	56.82 (±1.7)	40.08 (±1.7)	65.89 (±1.8)	35.14 (±1.9)	60.37 (±1.9)
Final length (mm)	47.29 (±1.5)	69.06 (±0.5)	44.75 (±1.3)	60.64 (±1.7)	47.02 (±1.3)	69.68 (±1.2)	39.82 (±1.5)	65.14 (±0.6)
IGR (Lenght)	0.0006	0.0004	0.0009	0.0003	0.0009	0.0003	0.0006	0.0004
RGR% (Lenght)	10.1048	7.0366	18.543	6.723	17.3154	5.752	13.3182	7.9013
SGR (Lenght)	0.0563	0.0398	0.0872	0.0334	0.0934	0.0327	0.0641	0.039
Initial weight (g)	7.38 (±0.3)	23.89 (±0.3)	6.66 (±0.3)	20.31 (±0.6)	6.11 (±0.3)	22.35 (±0.6)	3.61 (±0.2)	24.21 (±0.7)
Final weight (g)	10.96 (±0.3)	29.72 (±0.2)	9.36 (±0.3)	25.36 (±0.7)	9.21 (±0.3)	32.57 (±0.6)	6.19 (±0.2)	29.8 (±0.3)
IGR (Weight)	0.002	0.0011	0.0017	0.0011	0.0021	0.0019	0.0028	0.0011
RGR % (Weight)	48.5095	24.4035	40.5405	24.8646	50.7365	45.7271	71.4681	23.0896
SGR (Weight)	0.2028	0.112	0.1745	0.1139	0.2104	0.1931	0.2765	0.1065

plankton, is rapidly oxidised to nitrite and then to nitrate. Since nitrite is an intermediate product in oxidation and reduction events, its residence time in aquatic environments is shorter than in other nitrogen forms (Yaramaz 1992). In October 2013, it was observed that there was an increase of the amount of phytoplankton at both stations. Accordingly, it can be assumed that ammonium in the environment has been used by phytoplankton species, thus reducing the nitrite rate (Glibert et al. 2016). The phytoplankton species, which have been detected at low levels in September, increased their numbers in the second station in May 2014. We believe that the sampling moment at Station 2 in May 2014 coincided with the initial phase of the proliferation of the phytoplankton community.

Depending on water temperature, organic and inorganic substances in water may change and excess nitrate can be detected because of the oxidation of these substances (Karayucel & Karayucel 2000, Burgin & Hamilton 2007). An increase in nitrate amount can be observed due to the decrease in the feed consumption of sea bream and sea bass species grown in net cages in winter as well as to the accumulation of uneaten feed in the environment. It is thought that the increase in nitrate amount during the winter may be due to excess of food in cages as well as to the precipitation and wave movements. According to Giritlioğlu (1975) and Hastings et al. (2003), the solubility of nitrate with water is high. In regions where the organic pollution is intense and during the periods of heavy rain, the amount of nitrate increases significantly. It is known that nitrate dissolves easily in water due to the rainwater washing of the agricultural lands, which is a source of higher nitrogen intake in marine environment. It is known that the average precipitation falling in the region during the study has been above the standard precipitation levels. The observed nitrate concentrations in the water samples from the winter season could be related to increased rainfalls in the study area.

The dissolution of phosphorus in natural waters and the increased flow of dissolved phosphorus from the land to the sea with rainwaters result in an increase in phosphorus concentration in aquatic ecosystems (Kalemci 2015). In the present study, it is seen that the amount of phosphorus increases in the winter season when heavy precipitation is observed. Growth in aquatic organisms is generally directly proportional to the amount of phosphorus. Phosphorus multiplies plankton and, consequently, higher organisms increase in numbers and size (Sönmez et al. 2008). In this study, it was found that there was an increase in the phytoplankton counts in October and November, coinciding with the increased amount of phosphorus.

Chlorophyll-a concentration in water is an important index showing the eutrophic level; it is also used to express the biomass in the environment (Demirak 2006). The chlorophyll-a concentration is closely related to surface water temperature, salinity, water current regime, uplift and nutrient concentrations (Fidan 2011). Kucuksezgin et al. (1995) detected 0.03–0.70 mgm^{-3} chlorophyll-a in the Aegean Sea. Chlorophyll-a was detected between 0.09–0.26 mgm^{-3} in Güllük Bay (Demirak 2006). Our study showed that the chlorophyll-a values were higher than chlorophyll-a values determined in previous years in the same area. This can be explained by the continuous introduction of nutrients into the marine environment. The higher chlorophyll-a values with the increase of the water temperature in March–June can be interpreted as a result of the use of the nutrients accumulated during the whole winter by the primary producers.

The main reason for the high concentration of suspended solids in the samples in the autumn was the increased amount of phytoplankton during this season and the accumulation of pollutants caused by the winds blowing towards the sea. In addition, the net cages for fish farming in the study area are fed 5–6 times a day. Water temperature is an essential criterion to determine the amount of food to be given to caged fishes. During the winter months, the feeding of sea bream and sea bass fish, which are warm-water fish, decreases and the enterprises reduce the amount of food. This is thought to be the main reason for the reduction of the total amount of suspended solids during the winter months.

The presence of toxic species in the phytoplankton is an essential issue in aquaculture (Yurga et al. 2005). It has been observed that *Prorocentrum* species produce okadaic acid and its derivatives (Balmer-Hanchey et al. 2003) and mussels containing species such as *Gymnodinium* sp. may cause poisoning in humans when consumed (Terzi 2008). However, the amount of these species is low and they are not yet appreciated as a substantial risk potential.

Many studies demonstrated that the survival rate and growth of mussels are directly related to the temperature, salinity, total suspended matter, organic matter, inorganic matter and chlorophyll-a content in the water environment (Jones & Iwama 1991, Karayucel & Karayucel 1999, Ren & Ross 2005). Hindioğlu et al. (2001) reported that the best survival rate in mussels had been reported about mussels fed with *Chaetoceros calsiltrans*. In

this study, *Chaetoceros* species were detected in the environment during the 2nd period when a high survival rate was detected.

Chicarro et al. (2000) studied *Mytilus galloprovincalis* larvae on the Portuguese coast; they identified larval groups, length-frequency distributions and performed age determinations according to shell sizes identical with those in our study. In this study, it was determined that mussels reached marketing length (>50 mm) in 18 months. Mazzola et al. (1999) recorded that mussels reached a market size of 7 cm in 18–24 months. According to Yildiz & Lök (2005), mussels could be harvested by reaching the market size (>50 mm) in one year or a little longer, which is in agreement with our results. In our study, *M. galloprovincialis* had developed gonads and could start reproduction in December, and the process continued until June.

In conclusion, as study demonstrates that *Mytilus galloprovincialis* has a very good reproductive and growth potential in the conditions of the in the Güllük Bay Kazıklı Port and is a suitable species for harvesting in the conditions of mussel farming in this area.

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