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Growth and Survival Rate of Juvenile Sea Cucumbers (*Holothuria tubulosa*, Gmelin, 1788) at Various Temperatures

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Abstract

The effect of various water temperatures on growth and survival rate of juvenile sea cucumbers (*Holothuria tubulosa*) had been investigated in this research. Average weight of 20.48±2.33 g of juvenile sea cucumbers were collected from the coastal zones of Aegean Sea by scuba diving. After the adaptation period, juveniles were placed into glass aquaria which have 4 different sea water temperatures and researched for 45 days under laboratory conditions. Sea water temperatures of trial groups were selected among the minimum (winter) and maximum (summer) temperatures recorded at the natural habitat of *H. tubulosa* juveniles. Thus, 4 temperature group were selected as 15, 20, 25 ve 30°C. The sea cucumber juveniles were fed on dried and powdered brown algae as references in the literature. The best specific growth rate (SGR) was recorded as 0.288±0.02 % day^{-1} in 25°C group. The lowest SGR was recorded as 0.085±0.005 % day^{-1} in 30°C trial group while the negative specific growth rate (-0.03±0.02 % day^{-1}) was recorded in 15°C trial group. The aestivation observed in 30°C and hibernation in 15°C group would be a reason for such lower specific growth rates. The survival rate for all groups were 100% and the best growth performance was recorded in 25°C among others (P<0.05).

Keywords: Sea cucumber, *Holothuria tubulosa*, aquaculture, temperature, growth.

Farklı Sıcaklıklarda Deniz Hıyarı (*Holothuria tubulosa*, Gmelin, 1788) Genç Bireylerinin Büyüme ve Yaşama Oranı

Özet

Bu çalışmada, farklı su sıcaklıklarının deniz hıyarı (*Holothuria tubulosa*) genç bireylerinin büyüme ve yaşama oranları üzerine etkileri araştırılmıştır. Ortalama 20,48±2,33 g ağırlıktaki genç *H. tubulosa* bireyleri Ege Denizi kıyılarından serbest dalış yöntemiyle elle toplanmıştır. Toplanan bireyler adapte edildikten sonra, laboratuar ortamında 45 gün boyuncadeniz suyu bulunan cam akvaryumlarda denemeye alınmıştır. Deniz hıyarlarının dağılım gösterdiği bölgedeki yaz ve kış deniz sıcaklıkları (en düşük ve en yüksek) sınır olmak üzere 4 farklı deniz suyu sıcaklığında (15, 20, 25 ve 30°C) deneme grupları oluşturulmuştur. Deneme süresince deniz hıyarları kurutulmuş kahverengi alg ile beslenmişlerdir. Deneme süresi sonunda en yüksek spesifik büyüme oranı (SBO) 0,288±0,02 % gün^{-1} olarak 25°C de kaydedilmiştir. En düşük spesifik büyüme oranı 0,085±0,005 % gün^{-1} değeri ile 30°C'de görülürken, 15°C'de negatif yönlü (-0,03±0,02 % gün^{-1}) SBO kaydedilmiştir. Büyüme oranlarındaki düşük değerlerin 30°C'de gözlenen estivasyon (uyku) ve 15°C gözlenen hibernasyon davranışına bağlı olduğu düşünülmektedir. Tüm deneme gruplarında yaşama oranı %100 olup en iyi gelişim 25°C 'de tespit edilmiştir (P<0,05).

Anahtar Kelimeler: Deniz hıyarı, *Holothuria tubulosa*, yetiştiricilik, sıcaklık, büyüme

Introduction

Sea cucumbers are echinoderms that are mass harvested for human consumption, primarily in China, but they are availablein several areas of the world, including Mediterranean Sea (Cakli *et al.*, 2004; Lovatelli *et al.*, 2004; Toral-Granda *et al.*, 2008; Aydin, 2008; Purcell, 2010; González-Wangüemert *et al.*, 2011; Sicuro *et al.*, 2012). *H. tubulosa* is considered as a potential sources of pharmalogical compounds (Bordbar *et al.*, 2011; Schillaci *et al.*, 2013) that used in dietary supply, aphrodisiacs, HIV and cancer medicines (Chen, 2003). Also this species is an important holothurian for the oligotrophic and hypereutrophic environment (İşgören-Emiroğlu and Günay, 2007a, 2007b). Moreover, it is reported that *H. tubulosa* promotes organic matter recycling in *Posidonia oceanica* meadows (Costa *et al.*, 2014) and stabilizes the bacterial community in the sediment depending on feeding activity (Amon and Herndl,

1991). They also used for gilthead sea bream fishing with longliners during the summer. However, increasing demand from China and inadequate management of sea cucumber stocks in many countries have resulted in severe over fishing (Kuganathan, 2014; Bruckner, 2006; Conand, 2004; Lovatelli *et al.*, 2004). Similar situations threaten sea cucumber stocks also in Aegean Sea of Turkey. Over fishing of *H. tubulosa* and other Mediterranean aspidochirotid holothurians for commercial use by exporting companies is a serious problem in Turkey (Aydin, 2008; Aydin *et al*., 2011; Sicuro and Levine, 2011). This causes significant decrease in the number of species in natural beds due to its reproduction period which is in the summer times (Günay *et al*., 2011; Kazanidis *et al*., 2014; Mezali *et al*., 2014).

H. tubulosa as a deposit-feeder, ingests superficial sediment, feed on non-living detritus and associated microorganisms (Massin and Jangoux, 1976, Moriarty, 1982, Birkeland, 1988) and inhabits *Posidonia oceanica* meadows where it often occurs in dense populations and represents a significant part of the macrozoobenthic biomass of the Mediterranean Sea (Mezali, *et al.,* 2006; Costa *et al*., 2014). Most aspidochirotid holothurians follow the life cycle of pre-auricularia, early, mid and late auricularia and subsequent metamorphosis to the doliolaria (nonfeeding) stage before settlement, pentactula, juvenile and adult stages (Hamel and Mercier, 1996; Hamel *et al*., 2003; Laxminarayana, 2005; Asha and Muthiah, 2002; Asha and Muthiah, 2005).

Many countries have been focused on sea cucumber fisheries management to protect valuable sea cucumber stocks for providing a continuing and sustainable income for the fishermen (Twalibu and Mgaya, 2004; Uthicke,2004; Purcell *et al*., 2014) and there have been many studies on the sea cucumber aquaculture in order to enhance natural stocks. Purcell *et al*. (2012) reported sea cucumber species that are suitable for the culture and mentioned about the problems to be faced during their aquaculture. In order to rear sea cucumber under culture conditions, optimum physicochemical parameters should be known. Because any change in water quality parameters as temperature, pH, saturated oxygen, free carbondioxide, alkalinity and some other salts may affect the growth and maturity of aquaculture species (Nikolosky, 1963, Jhingram, 1985, Iwama *et al*., 2000). As reported by the previous studies, water temperature is one of the most important physical factor affecting the metabolism (Yang *et al*., 2005), growth and physiological performance of the sea cucumber (Dong *et al*., 2005). There are many studies on effects of temperature on tropical and cold water sea cucumber present in the literature (Yu and Song, 1999; Dong *et al*., 2005; Yang *et al*., 2005; An *et al*., 2009; Lavitra, 2010; Zamora and Jeffs, 2012). The biggest problem for culture of sub-tropical species as Mediterranean ones is the lack of information about optimal rearing conditions. This study aims to reveal the optimum temperature range for rearing of sea cucumber *H. tubulosa* under laboratory conditions.

Materials and Methods

Holothuria tubulosa, a member of Holothuroidea classis and wide spread in Mediterranean and Aegean Sea coasts were researched. *H. tubulosa* juveniles (<30 g) were collected from Urla (38°21'48.4"N 26°46'27.1"E), Karaburun (38°38'12.5"N 26°31'25.2"E) and Çeşme (38°18'56.4"N 26°22'54.2"E) coasts of İzmir city of Turkey by scuba diving (Figure 1).

Collected samples were immediately transferred

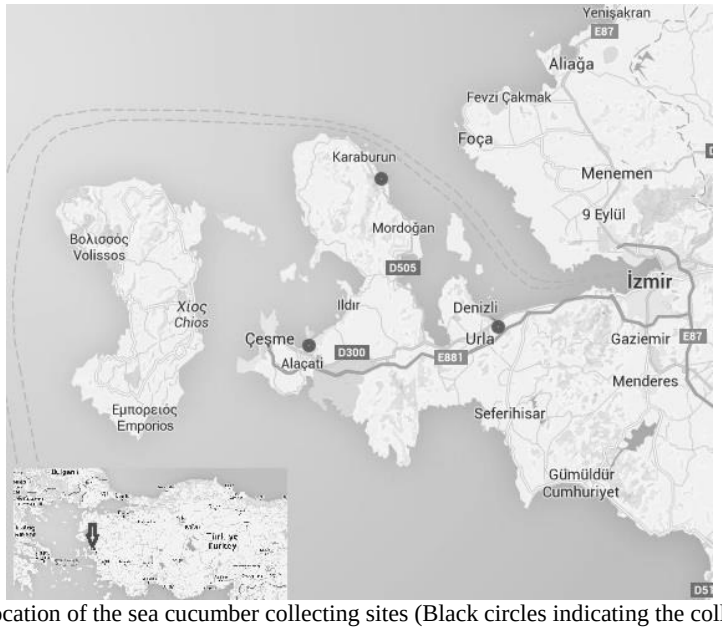

Figure 1. Geographic location of the sea cucumber collecting sites (Black circles indicating the collection sites).

to Ege University, Faculty of Fisheries, H. Okan Kamacı Aquaculture Research Unit in Urla and acclimated in 2x2x1 m tanks for 30 days under running sea water and fed by dried brown macroalgae. The juvenile sea cucumbers without any visual defect on body texture were selected, put into bare aquaria without any sediment and starved for 24 h prior to the weighing in order to let sea cucumbers to empty sand, feed and feces in their digestive tracks as indicated by(Slater *et al*., 2009). The wet weighing procedure of was followed as specimen were taken out of water and placed on a dry sponge for 1 min to remove external water (Battaglene *et al*., 1999). Then, weighed to the nearest gram on digital balance (OHAUS, Scout Pro). Selected juveniles with a mean weight of 20.48±2.33 g (min.15.8- max.24.4) were randomly placed into 60 (l) x 50 (w) x 40 (h) cm glass aquaria by 5 individual per aquarium.

The experimental temperatures as 15°C, 20°C, 25°C and 30°C were selected among the temperature range based on the distributional limits of collected sea cucumbers(Yang *et al*., 2005). The seawater temperatures were referred from the records of Turkish State Meteorological Service (TSMS, 2014) and own preliminary measurements on the selected sites. Seawater temperature trial groups had been set with three replicates for each.

All trial aquaria were filled with natural quartz sand as sediment by 5 cm depth. The sediments were sieved using a 1mm mesh to remove large particles and frozen at -21°C to kill meiofauna and rinsed by fresh water prior to placement (Kitano *et al*, 2003). Mechanically filtered seawater taken from the adjacent sea was used in all experiments. One-third of the water in all aquaria was changed with pre-heated seawater for every three days. Saturated oxygen level was monitored daily by digital oxygen-meter (Hanna HI 9142) and kept in the range of 7.2 and 8.5 mg L^{-1} by continuous aeration through air stones. pH level was in the range of 7-7.8 and monitored daily by pH-meter (Hanna HI 98103). The salinity in all experiment aquariums changed between 38-42 ppt and monitored by handheld refractometer (Atago Smill Refractometer) daily. The light regime was 8h l:16 h d which was provided by 4x40 W fluorescent light and controlled by timers. The seawater temperatures were set in desired level by in-water type thermostatic heaters (Atman, 500W) in each aquarium. The temperature level increased to desired level at a rate of 2°C day^{-1} and juveniles kept at adjusted temperature for 5 days prior to the experiment in order to acclimatize.

The juvenile sea cucumbers fed on dried and powdered brown macroalgae mixture (50% *Cystoseira barbata*, 50% *Sargassum vulgare*) by 3% of total biomass, daily. The seaweeds are natural feeds for sea cucumber juveniles in post-larval rearing stage. Although, the success of artificial feeds as Algamac in promoting growth of a tropical sea cucumber *Holothuria scabra* has been reported by Giraspy and Ivy (2008), the efficiency on growth of *Holothuria tubulosa* is not well known and commercially availability is limited in Turkey. Thus, a natural macroalgal diet which is successfull in post-larval and nursery rearing stages (Asha and Muthiah, 2007; Liu *et al*., 2010; Yanagisawa, 1998) had been used as feed in this study. The proximate composition of macroalgal diet was analyzed Wendee Analyse Method (Nehring, 1960) and listed in Table 1.

Specific growth rate (SGR) and Survival rate (SR) were calculated as an indicator of growth performance among treatment groups. Specific growth rate (SGR) and Survival Rate (SR) were calculated as follows:

$$SGR\ (\%\ \mathrm{d}-1) = \frac{(\ln Wf - \ln Wi)}{t}\ x\ 100$$

$$SR\ (\%) = \frac{Nf}{Ni}\ x\ 100$$

where, W*i* and W *f*are initial and final body wet weight of sea cucumbers in each aquarium; t istime of trial in days, N*f* and N*i* are the final and initial number of individuals in each aquarium.

All data were analyzed using descriptive statistics and compared by one-way ANOVA. Normality was determined by Kolmogorov–Smirnov test and homogeneity of variance was analyzed by Levene's test. Duncan's multiple range test was used for post hoc comparison of the means. Data were presented as mean ±SD where means were considered significantly different at $P<0.05$. All statistical analyses were performed by SPSS 15.00software.

Results

Physicochemical Parameters

During the experiment period pH values kept among the range 7-7.8 which is ideal for sea cucumber breeding (Figure 2). There were no significant differences for pH values among the treatment groups ($P>0.05$). Saturated oxygen amount was between 7.2 and 8.5 mg L^{-1} for all groups without

Table 1. The proximate composition of macroalgal diets

Species	Crude Matter (%)	Crude Cellulose (%)	Crude Lipid (%)	Crude Ash (%)	Crude Protein (%)	Carbohydrate (%)
Sargassum vulgare	90.31	38.40	0.01	15	5.52	32.30
Cystoseira barbata	90.77	6.22	0.01	15	5.57	63.97

any significant difference (P>0.05). Salinity levels showed a simultaneous increasing trend due to the evaporation in all groups but no significant difference had found among groups throughout the experiment period (P>0.05) (Figure 3). Average salinity levels were 38.13±1.37; 39.46±1.50; 40.68±1.99 and 41.97±1.87 ppt at 15, 20, 25 and 30°C treatment groups, respectively.

Mean Body Weight, Specific Growth Rate and Survival Rate

There were significant differences in mean body weights of juveniles among groups (Figure 4). The best growth was determined at 25°C (P<0.05). On the other hand at 30°C, the body weight started to decrease from 22.74±4.81 g to 22.18±16.73 g on the30th day of the experiment. Also, at 15°C (Control), bodyweight decreased from 22.48±3.83 g to 19.81±2.96 g at the last 30 days of the experiment.

Specific growth rate (SGR) also showedits highest value at 25°C and negative value at 15°C (P<0.05) (Figure 5). The SGR valuesrecorded as 0.244±0.04; 0.288±0.02; 0.085±0.005 and -0.03±0.02 % day^{-1} at temperature 20, 25, 30 and 15°C (Control), respectively. The maximum SGR was determined as 0.288±0.02 % day^{-1}at 25°C. Survival rate (SR) was 100% for all groups.

Discussion

Recently, popularity of healthy human food has started increasing demand for sea cucumber as much as other considerably natural food recourses. On the other hand, landing of wild sea cucumber have declined continuously due to over exploitation (Conand, 2004; Uthicke, 2004). Therefore, researches on sea cucumber rearing especially in the Far East and Asian countries has been considered more and more. There are plenty of studies focused on physicochemical parameters effects on growth of tropical sea cucumbers (Hamel and Mercier, 1996; Kashenko, 2002; Asha ve Muthiah, 2005; Wang *et al*., 2008; Hu *et al*., 2010). One of the most physicochemical parameter that effecting growth of sea cucumberis the temperature (Dong *et al*., 2006).

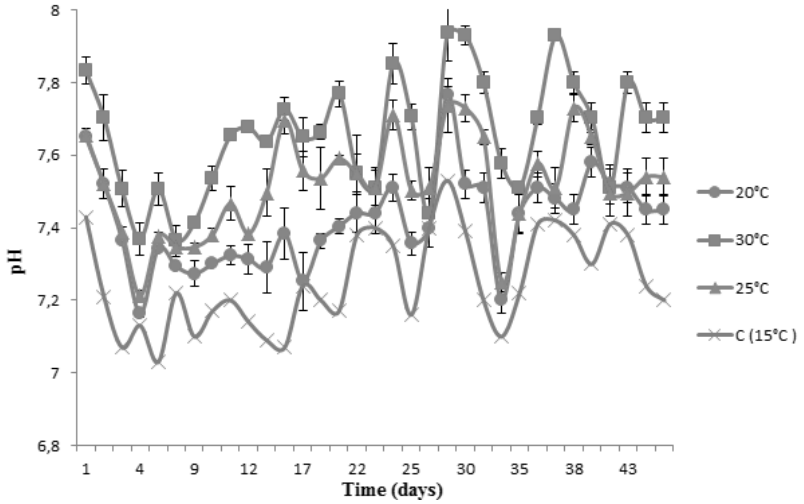

Figure 2. Changes in pH values (mean±SD).

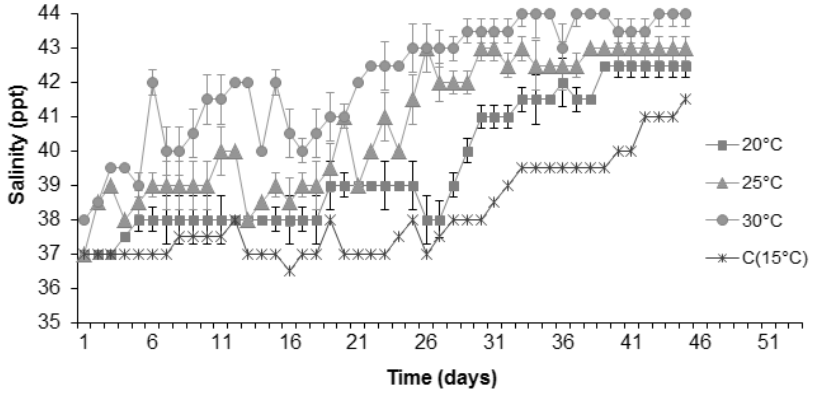

Figure 3. Changes in salinity values (mean±SD).

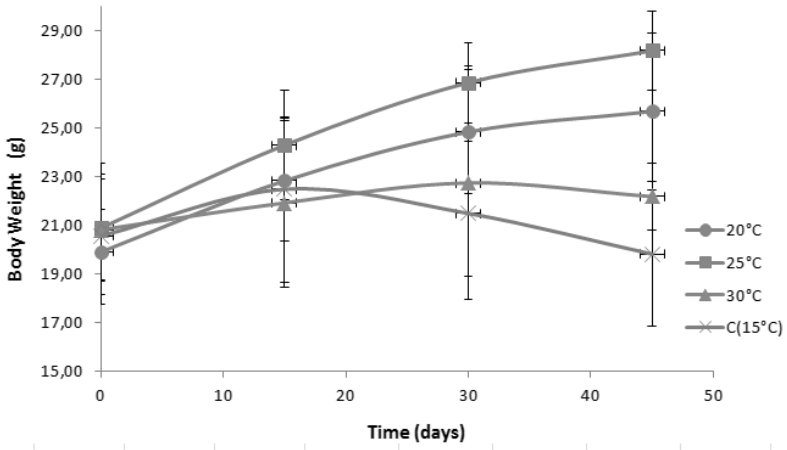

Figure 4. Changes in body weights of the sea cucumber juveniles in all groups (mean±SD).

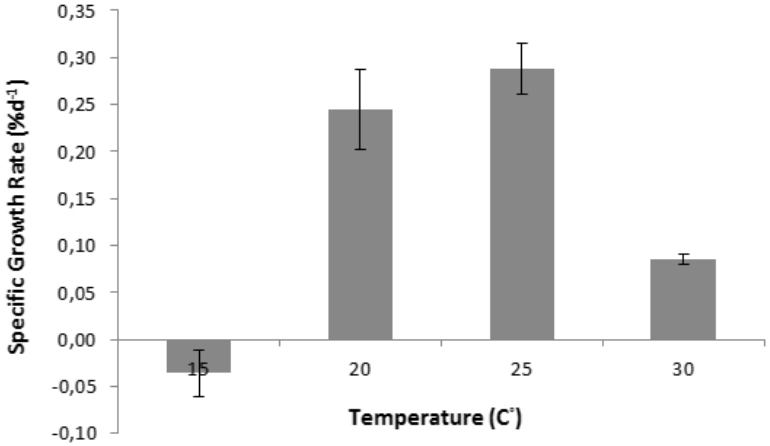

Figure 5. Specific growth rate (SGR) of *Holothuria tubulosa* juveniles at different temperatures for 45 days.

However, studies on temperature as a determinant in terms of effecting growth and metabolism are limited and focused on tropical sea cucumbers. The spawning temperature of *H. tubulosa* is the sole knowledge so far about the temperature effect in the culture of the species (Despalatovic *et al*., 2004; Günay *et al*., 2011). Yet, there is no evidence about the temperature effect on the growth or aestivation for *H. tubulosa*. In this study *H. tubulosa* showed the best growth rate at temperature 25°C. There was also a slight increase in weight measurements at 20°C. A temperature below and above optimal rates could result in declined and raised metabolic rate, respectively, while affecting respiratory, feeding and assimilation rate (Landau, 1992). The previous studies reported that optimal temperature for growth is species-specific and in consistent to this study, feeding activity reduced outside temperature tolerance range. In several studies, optimumtemperature for tropical species as *Holothuria atra* and *Holothuria scabra* were reported over 20°C (Seeruttun *et al*., 2007; Lavitra *et al.*, 2010) while optimum temperature for cold water species as *Apostichopus japonicus* and *Stichopus japonicas* were reported below 20°C as 15-18°C for *Apostichopus japonicus* (Yu and Song, 1999; Asha and Muthiah, 2005) and 5-15°C for *Stichopus japonicus* (Chen, 1990).

Aprevious study on temperature effects on growth-ration reported that SGR value decreased above and below the optimum temperature (An *et al*., 2007, 2009). Similarly, in this study, SGR decreased in temperatures other than 20-25°C. The highest SGR in this study was lower compared to other studies on sea cucumber (Yang *et al*., 2005; An *et al*., 2009; Liu *et al*., 2010; Seo and Lee, 2011). Characteristics of the species, weights of the sea cucumbers juveniles and food types might be the basis for the differences between this study and the others. In this study, all groups fed on the same natural feed (Battaglene *et al*., 1999; Liu *et al*., 2010) that protein and fat values were lower than the artificial feeds. Seo and Lee (2011) reported that SGR of sea cucumbers were significantly affected by dietary protein and lipid levels. It was found by Sun *et al*. (2004) that, *A. japonicus* showed an increase in growth rate with the protein content contained in the formulated feeds and

the highest growth rate was observed when they were fed with 21.5% protein. Previous studies for *S. japonicus* showed that better growth is occurred when fed on diets containing 18.21-24.18% protein (Zhu *et al*., 2005) and the highest growth rate was observed when they were fed with 33% protein (Seo *et al*., 2011). Chu Yuan kee and Appadoo (2007) reported that *Bohadschia marmorata* fed with a supplement of protein showed a better growth. Previously tested sea cucumbers were tropical species, but Günay (2012) reported inthe study about digestive enzyme activities that feeding *H. tubulosa* with feed containing high protein when cucumber is active phase was suitable for aquaculture.

The minimum and negative SGR occurred at 30 and 15°C, respectively. At both 15 and 30°C, along period of burrowing and inactive behaviors were determined by 24 hours camera record. Four phases of the annual cycle in *A. japonicus* have been reported by Choe (1963) an active phase, during which the animal is active; a prophase of aestivation, during which food consumption decreased gradually; a deep aestivation phase, during which the animal is idle; and a reversion phase, during which the animals recover to pre-aestivation activity. At 30°C a drop of body weight was occurred after day 30. Besides, little or no feces were removed from aquariums daily. It is reported by Gao *et al*. (2009) for *A. japonicus* that there is no increase in weight when entered the prophase of aestivation on Day 40 at 21°C. Yang *et al*. (2005) also reported that, in large and some medium sea cucumbers (73.3–139.3 g), daily food consumption decreased to zero after 11–21 days at 25°C. Therefore, these sea cucumbers were considered as being in enter the aestivation phase. At 15°C, decrease in weight also occurred and no feces were found in the aquariums after 15 days of experiment. Sea cucumbers were observed to be in the hibernation phase after the day 15 at 15°C. Therefore, as soon as the samples entered the prophase of hibernation, feed consumption decreased gradually. A previous study about *A. japonicus* also reported a drop of metabolic rate which directly affect feeding during hibernation or aestivation (Bao *et al*., 2010). The results of this study showed that sea cucumbers in all groups exhibited excellent survival rate (100%) during the study period. Several authors have reported that temperature had significant effects on survival rate and also associated with salinity. 100% survival rate was reported at 23, 27, 25°C for normal salinity of sea water for *A. japonicus* (Hu *et al*., 2010). Chu Yuan kee *et al*. (2007) found that, *B. marmorata* displayed 100% survival rate at salinity 25 and 35 ppt and 91,67% and 66,65% survival rates in temperature 28 and 32°C, respectively. The reason of the excellent survival rate of this study might be the optimum physicochemical parameters and conditions for *H. tubulosa*. İşgören-Emiroğlu and Günay (2007b), also reported that *H. tubulosa* survived in a wide range of physicochemical parameters. Stocking density had also significant effect on survival rate (Battaglene, 1999; Dong *et al*., 2010). Stocking density of this study was optimum for survival according to Dong *et al*. (2010).

In conclusion, this study showed that optimum growth temperature for *H. tubulosa* was between 20-25°C for the samples collected from the sea with the mean weight of 20.48±2.33 g. Little or no growth was seen at 15°C and 30°C depending on the hibernation and aestivation. However, considering the present data, further studies will be needed for the optimum dietary protein and lipid levels for growth of juvenile *H. tubulosa*, which is the most common ones and have been exported to the world from Turkey. In addition, further investigation could be carried out with the different sample sizes as a second variant near the temperature. Thus, basic knowledge for establishing a culture stock for sea cucumbers in the Mediterranean region would be provided.

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