


RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

Effects of various stocking densities on hatching and survival rates of sea cucumber *Holothuria tubulosa* eggs (Gmelin, 1788)

Farklı stok yoğunluklarının deniz hıyarı *Holothuria tubulosa* (Gmelin, 1788) yumurtalarının açılma ve yaşama oranlarına etkileri

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Abstract: In this study, it was aimed to determine the opening and survival rates of *Holothuria tubulosa* eggs at four different stocking densities (1,5,15,30 eggs / ml) and to determine the effect of stocking density on egg size and larval size in different stages. Fertilized *Holothuria tubulosa* eggs were stocked in cylindrical conical collectors with a capacity of 30 l. Eggs were counted under light microscope at fertilization, early gastrulation, late gastrulation and hatching stages. It was found that there is an inverse relationship between stock intensity and opening rate ($r = -0.848$; $p < 0.001$). The lowest hatching and survival rate was determined in 30 eggs / ml group ($p < 0.05$). However, different stocking densities did not affect the diameter of the egg in different embryological stages ($p > 0.05$) and the length of early auricularia larva at 65 h ($p > 0.05$). As a result, the optimal stocking density for the highest hatching and survival rates of the fertilized eggs of *Holothuria tubulosa* was determined as 1-5 eggs / ml.

Keywords: Fertilized egg, hatching rate, *Holothuria tubulosa*, sea cucumber, stocking density, survival

Öz: Bu çalışmada, *Holothuria tubulosa* yumurtalarının, dört farklı stok yoğunluğunda (1,5,15,30 yumurta/ml) açılma ve yaşama oranlarını belirlemek ve aynı zamanda stok yoğunluğunun farklı evrelerdeki yumurta çapına ve larval boyutuna etkisini ortaya koymak amaçlanmıştır. Döllenen *Holothuria tubulosa* yumurtaları, 30 l hacimli silindirik konik kollektörlere stoklanmıştır. Yumurtalar; döllenme anında, erken gastrulasyon, geç gastrulasyon ve yumurtadan çıkma aşamasında mikroskop altında sayılmıştır. Stok yoğunluğu ile açılma oranı arasında ters ilişki olduğu bulunmuştur ($r = -0.848$; $p < 0.001$). En düşük açılma ve yaşama oranı 30 yumurta/ml stoklandığı deneme grubunda saptanmıştır ($p < 0.05$). Ancak farklı stok yoğunlukları farklı embriyolojik safhalardaki yumurtanın çapını ($p > 0.05$) ve 65. saatteki erken auricularia larvasının boyunu ($p > 0.05$) etkilememiştir. Sonuç olarak, *Holothuria tubulosa*'nın döllenmiş yumurtalarının en yüksek açılma ve yaşama oranları için optimum stoklama yoğunluğu 1-5 yumurta / ml olmuştur.

Anahtar kelimeler: Döllenen yumurta, açılma oranı, *Holothuria tubulosa*, deniz hıyarı, stok yoğunluğu, yaşama oranı

INTRODUCTION

Fresh and processed sea cucumbers (beche-de-mer) are being used as luxury food and food supplement for several centuries (Asha and Diwakar, 2013; Choo, 2008). Increasing demand for beche-de-mer all over the world has been caused excessive fishing and extinction of natural stocks (Purcell et al., 2013). Depending on the movement and feeding activities of sea cucumbers which is resulting as regulation of both oligotrophic and hypereutrophic environments (İşgören-Emiroğlu and Günay, 2007), extinction of these species may reversely affect the recovery process of marine sediments.

Aquaculture studies on sea cucumber species have been carried out in the Far East and Asian countries for several years (Gamboa et al., 2005, Tuwo and Tresnati, 2015). The amount of sea cucumbers provided by aquaculture has been increased by six times between the years 2003 and 2015, while catch amount has been stabilized during the last five years. FAO (2017) has reported 208104 tons and 43216 tons of sea cucumber production from aquaculture and fisheries for 2015, respectively. *Holothuria scabra* is the most valuable (Raison, 2008) and the most commonly cultured tropical sea cucumber species (Purcell et al., 2012) followed by *Apostichopus japonicus*, which is the other sea cucumber species widely produced in all over the world. However, many studies on aquaculture of new sea cucumber species are being conducted on all over the world (Santos et al., 2015, Sicuro and Levine, 2011; Tolon et al., 2017).

Holothuria tubulosa is one of the most common and commercial sea cucumber species in the Mediterranean Sea (Ocaña and Tocino, 2005) and a candidate for aquaculture (Tolon et al., 2017). They are extremely demanded due to their rich proximate composition, high protein component and nutritional value (Çakli et al., 2004). Sicuro and Levine (2011), reported that *H. tubulosa* might be one of the potential aquaculture species in the Mediterranean Sea. Recent studies on adaptation of *H. tubulosa* to aquaculture reported successful results (Günay et al., 2015; Tolon et al. 2015). However, studies on the hatching stages are lacking in the literature.

Stocking density is the most important criteria at hatching stage for production of sea cucumbers (Asha and Diwakar, 2013; Liu et al., 2010; Battaglione and Bell, 1999). Previous studies on *H. scabra* (Asha and Diwakar, 2013), *Holothuria scabra versicolor* (Ivy and Giraspy, 2006) and *A. japonicus* (Liu et al., 2010) reported a significant relationship between stocking density and hatching rate. This is the first report in the literature which aims to determine the optimum stocking density of *H. tubulosa* eggs for maximum hatching and survival rate. Therefore, the hatching and survival rates of fertilized sea cucumber *H. tubulosa* eggs under various stocking

densities have been investigated. Moreover, the effects of various stocking densities on the diameter of eggs and larvae size in early auricularia stage have been determined.

Therefore, we investigated the hatching and survival rates of fertilized sea cucumber *H. tubulosa* eggs under selected stocking densities. Moreover, we determined the effects of various stocking densities on the diameter of eggs and larvae size in early auricularia stage.

MATERIAL AND METHODS

H. tubulosa adults were hand-picked from the Aegean Sea shores of the Ildir village in Izmir city, Turkey (38°23'48.61"N- 26°28'26.02"E) by scuba-divers. Thirty brooders (150±15 g) were induced to spawn by raising water temperature 3-5 °C above initial temperature of 23 °C in 1000 l PVC tanks (Battaglione et al., 2002). Since gender of sea cucumbers can not be distinguished from their physical characteristics, only one male continuing to spawn left in the tank. Thus, excessive sperm density in the tank could be prevented. After spawning of females, samples were taken from the water column into a 500 ml beak, the fertilization rate was checked under the microscope and the egg diameters were recorded (at 0 h). The fertilized eggs in the water column were then collected in a 70 l collector, by siphoning water through a 80 µm sieve and washed to remove excess sperm. Five subsamples were taken from 70 l collector and counted to estimate average density.

The trial was consisted of four stocking densities, selected as 1 (Group A), 5 (Group B), 15 (Group C) and 30 (Group D) eggs/ml. Fertilized eggs were transferred to 30 l trial collectors. Five subsamples were taken from each collector, than counted and diameters of eggs were measured under microscope (1st hour).

Trial was carried out in three 30 l cylinder collectors which were covered by 80 µm sieve and placed into one 1000 l PVC tanks (Figure 1). Gentle water circulation by continuous flow of filtered sea water was applied. Dissolved oxygen concentration was maintained at 7.6±0.3 mg/l by continuous air flow through the air diffusers in all collectors, water temperature and pH were 23.5±0.2 °C and 7.4 ± 0.1, respectively. Black covers were placed on top of each tank in order to keep the eggs in dark environment.

The development of fertilized eggs was monitored under the microscope every 4 hours to detect the accurate time for sampling that majority of them are at the same development stage. Initial survival rates (about in one hour), early gastrulation (20th hour), late gastrulation (45th hour) and hatching rates at early auricularia larvae stage (65th hour) have been determined for all groups. The time of sampling was determined when the majority of eggs (≥90%) were at the same stage in all groups. Egg and larvae samples were taken from

the water column after pausing the aeration for 1 minute. Egg diameter and larvae size were measured by a graduated micrometer eyepiece and compound microscope at 10X magnification. Larvae size was measured from prominent posterior protrusion (p) to anterior commissure (a) (Figure 2). Five 1 ml subsamples were taken from each collector and counted using a Sedgewick-Rafter chamber under a microscope to calculate survival and hatching rate at all sampling times.

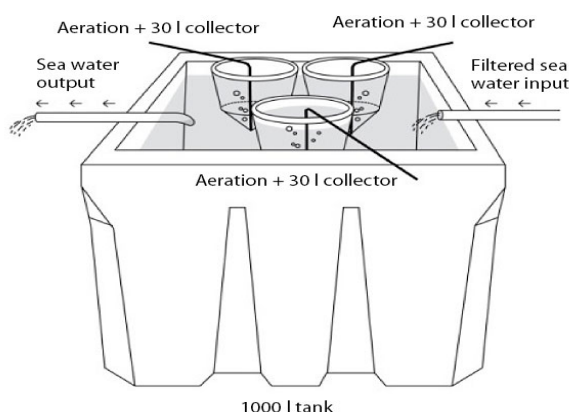


Figure 1. Design of the experimental tank

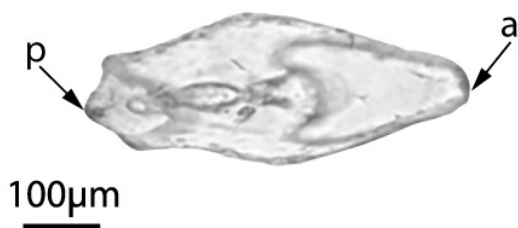


Figure 2. Early auricularia larvae of *H. tubulosa*; posterior protrusion (p), anterior commissure (a)

Table 1. Size of fertilized eggs (1st, 20th and 45th hours) and auricularia larvae (65th hour) at stocking density groups (mean±SE, n=15)

Hour	Size (μ)			
	Group A	Group B	Group C	Group D
1	252.85±3.30	255.34±6.31	252.14±1.13	252.89±3.65
20	253.64±2.41	253.64±3.57	245.48±2.89	247.52±2.32
45	398.48±4.61	405.96±3.75	391.68±7.09	390.32±4.03
65	492.32±5.58	493.68±4.85	480.08±7.21	496.40±4.18

Groups labels indicate: A=1 egg/ml; B=5 eggs/ml; C=15 eggs/ml; D=30 eggs/ml

Statistical analysis

The data were tested for homogeneity of variance using Levene's test prior to analysis. Normal distribution of the data was tested by Kolmogorov-Smirnov. Arcsine transformation was performed to the data of survival and hatching rates before analysis.

Survival rates (20th and 45th hours), hatching rates (65th hour), egg (1st, 20th and 45th hours) and larvae size (65th hour) were compared among the trial groups. Data of the groups were analyzed by one-way ANOVA and significant differences between treatments were determined by Duncan's Multiple Range test.

Level of 0.05 was accepted as an indication of statistical significance. Nonparametric Spearman Correlation Analyses were performed to determine the relationship between stocking density and hatching rate. Temperature, pH and dissolved oxygen values were presented as mean ± standard deviation (SD). Size of samples, survival and hatching rates were presented as mean ± standard error (SE).

RESULTS

Male sea cucumbers started to spawn in the afternoon (16:00-18:00), and females followed them approximately one hour later. Elongations were observed for the measured eggs for all groups after the early gastrulation. More than 90% of the population in all collectors reached early gastrulation, late gastrulation and early auricularia stages at 20th, 45th and 65th hours, respectively.

During the study, dead or deformed eggs and larvae stayed very close to the bottom of the tanks in each collector. There were no significant differences between eggs and larvae sizes of all groups (A, B, C, D) per sampling times ($p > 0.05$) (Table 1). No differences found in sizes among all treatments but there were significant differences in hatching rates ($p < 0.05$). The lowest hatching rate was observed in group D at 30 eggs/ml stocking density. An inverse relationship was detected between stocking density and hatching rate according to the results ($r = -0.848$; $p < 0.001$). Hatching rates were 81.1 ± 1.11 , 81.6 ± 1.22 , 53.3 ± 0.39 and $43.1 \pm 0.95\%$ at stocking densities 1, 5, 15 and 30 eggs/ml, respectively (Figure 3).

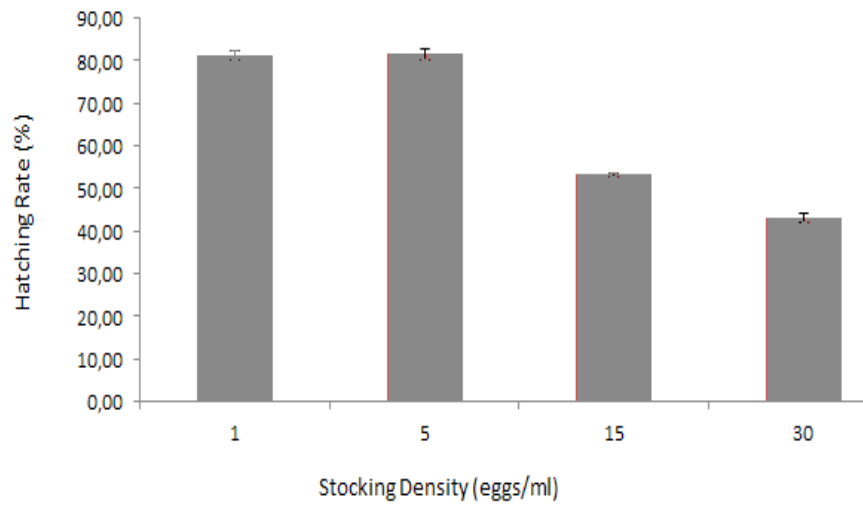


Figure 3. Effect of stocking density (eggs/ml) on hatching rates (%) of fertilized *H. tubulosa* eggs

There were no significant differences for survival and hatching rates of group A and B at all sampling times ($p > 0.05$). There were significant differences in survival and hatching rates between group C and D at

all sampling times ($p < 0.05$). Group D had the lowest survival rates for all sampling times (Table 2). The stocking density determined as to be maximum 5 eggs/ml for fertilized *H. tubulosa* eggs in this study.

Table 2. Survival (1st, 20th and 45th hours) and hatching rate (65th hour) at eggs incubation time in stocking density groups (mean \pm SE, n=15)

Hour	Survival and Hatching Rates (%)			
	Group A	Group B	Group C	Group D
1	100	100	100	100
20	94.4 \pm 5.55 ^a	97.4 \pm 1.37 ^a	59.8 \pm 1.03 ^b	46.7 \pm 1.43 ^c
45	94.4 \pm 4.56 ^a	92.1 \pm 0.10 ^a	59.6 \pm 0.68 ^b	44.7 \pm 0.50 ^c
65	81.1 \pm 1.11 ^a	81.6 \pm 1.22 ^a	53.3 \pm 0.39 ^b	43.1 \pm 0.95 ^c

Groups labels indicate: A=1 egg/ml; B=5 eggs/ml; C=15 eggs/ml; D=30 eggs/ml. Data with different superscripts in rows are significantly different from each other ($p < 0.05$)

DISCUSSION

The significant relationship between stocking density and hatching rate for sea cucumber species *Holothuria scabra* (Asha and Diwakar, 2013; James, 1996) and *Apostichopus japonicus* (Sui, 1989; Yanagisawa, 1998) was mentioned in the previous studies. Conforming to the results of these studies, it was found a significant inverse relationship between stocking density and hatching rates in the groups with high stocking density (groups C and D) for *H. tubulosa* eggs in this study. Previous studies on echinoderms reported that gastrula stage is one of the most vulnerable phase to external factors as salinity, temperature and heavy metal ions (Yaroslavtseva et al., 2002; Kashenko, 2005; Pia et al., 2012). Similarly, mortalities were first seen in early gastrulation in this study especially in high stocking rates,

groups C and D. Dissolved oxygen, pH and temperature parameters were kept at optimum levels during the study. Big differences in temperature avoided as described by Agudo (2016), pH and dissolved oxygen were also kept in optimum range (Agudo, 2016; James et al., 1994).

Therefore, low survival rate caused by high stock density observed at the first development stage. The results of this study advising low stocking densities like 1-5 eggs/ml for successful breeding of *H. tubulosa*. These stocking densities reported in this study is similar with the findings of Ramofafia et al. (1995) (2.7 eggs/ml for *Holothuria atra*), Agudo (2006) (0.3-1 eggs/ml for *Holothuria scabra*) and Guisado et al. (2012) (3 eggs/ml for *Athyonidium chilensis*).

Similar with the report of Pitt et al. (2001), precise-

ly cleaned and gently handled fertilized eggs can be incubated up to 5 eggs/ml, under the determined temperature, dissolved oxygen and pH levels of this study. In contrast, Asha and Diwakar (2013) reported the highest (66.4%) and lowest (22.6%) hatching rates at 0.5 eggs/ml and 6 eggs/ml stocking densities for *H. scabra* eggs, respectively. In this study high hatching rates were observed for *H. tubulosa* eggs in both low stocking density groups (1 and 5 eggs/ml). Therefore, it can be concluded that stocking densities up to 5 eggs/ml do not critically influence the hatching rates of *H. tubulosa* eggs in this study.

Liu et al. (2010) reported over 80% hatching rate and no significant differences among 0.2, 0.5, 1, 2, 5 and 10 eggs/ml stocking densities groups where the significant differences appeared at 20 and 50 eggs/ml stocking densities for *Apostichopus japonicus*. Similarly, hatching rates at 15 and 30 eggs/ml stocking densities were found lower than the other density groups for

H. tubulosa, in this study.

In conclusion, selected stocking densities had no significant effect on hatching time and size of *H. tubulosa* eggs in this study. Although, synchronized embryonic development stages observed in all groups, high mortality and low hatching rates recorded at high stocking density groups like 15 and 30 eggs/ml. The stocking density of 1-5 eggs/ml is determined as optimum and strongly suggested for highest hatching and survival rate of fertilized sea cucumber *H. tubulosa* eggs

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