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SHORT PAPER

Effect of Sediment Grain Size on Growth Performance of Juvenile Sea Cucumber (*Holothuria tubulosa*)

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Abstract

It is important to know the impact of sediment type on feeding and growth performance of sea cucumbers in aquaculture which is getting more importance due to the critical decrease in valuable stocks in all seas. The purpose of the present study is to investigate the effect of grain size on growth performance of juvenile sea cucumber (*Holothuria tubulosa*) under aquaculture conditions. In the present study, the growth performance of juvenile sea cucumber (*H. tubulosa*) on 3 different grain sized sediment has been investigated in laboratory conditions for 61 days. Sea cucumber juveniles (35.02±0.52 g) were placed into the 200x50x50 cm aquaria by 350 g m^{-2}. Natural crushed quartz sediment in different grain sizes (1 mm, 3 mm, 7mm and 0.2 mm (control)) were placed inaquariaby 10cm height. Final mean weight of the juveniles in sediment groups1 mm, 3 mm, 7 mm and 0.2 mm (control) were recorded as 49.18±0.94 g, 44.23±0.35 g, 40.72±0.53 gand 39.57±0.50 g, respectively. Specific growth rate of juveniles among sediment groups were ranged between 0.20% day^{-1} (Control) and 0.53 % day^{-1} (1mm). Overall, best growth performance was found as significant in 1 mm sediment group.

Keywords: Sea cucumber, sediment grain size, growth, *Holothuria tubulosa.*

Sediment Tane Boyutunun Deniz Hıyarı (*Holothuria tubulosa*) Genç Bireylerinin Büyüme Performansı Üzerine Etkileri

Özet

Dünya denizlerinde değerli deniz hıyarı stoklarındaki kritik azalmalar nedeniyle gittikçe önem kazanan deniz hıyarı yetiştiriciliğinde kullanılan sediment tane boyutunun, beslenme ve büyüme performansı üzerindeki etkilerinin bilinmesi önemlidir. Bu çalışmanın amacı tane boyutunun deniz hıyarı (*Holothuria tubulosa*) juvenillerinin büyüme performansı üzerindeki etkilerinin incelenmesi olarak belirlenmiştir. Bu çalışmada, denizhıyarı (*H. tubulosa*) genç bireylerinin 3 farklı sediment üzerinde büyüme performansları laboratuvar koşullarında 61 gün süreyle incelenmiştir. Ortalama 35,02±0,52 g ağırlıkta deniz hıyarı genç bireyleri 200x50x50 cm ölçülerindeki cam akvaryumlara 350 g m^{-2} stok yoğunluğunda yerleştirilmiştir. Farklı boyutlarda (1 mm, 3 mm, 7 mm ve 0,2 mm (kontrol)) doğal kırma kuvartz sediment tüm akvaryumlara 10 cm yüksekliğinde serilmiştir. Grupların son ortalama ağırlıkları 1 mm, 3 mm 7 mm ve kontrol grupları için sırasıyla 49,18±0,94 g, 44,23±0,35 g, 40,72±0,53 g ve 39,57±0,50 g olarak kaydedilmiştir. Spesifik büyüme oranı sediment gruplarına göre 0,20 % $gün^{-1}$ (kontrol) ila 0,53% $gün^{-1}$ (1 mm) arasında değişmiş. Tüm değerlendirmelerde 1 mm sediment grubundaki büyüme performansı en iyi düzeyde bulunmuştur.

Anahtar Kelimeler: Deniz hıyarı, sediment tane büyüklüğü, büyüme, *Holothuria tubulosa.*

Introduction

Sea cucumbers, also referred as earth-worms of the sea, are important organisms of marine benthic ecosystems. Holothurians can assimilate low content of organic matter as live diatoms, bacteria and detritus by passing sediment through their gut system (Moriarty, 1982; Uthicke, 1999; Yingst, 1976). By feeding and movement characteristics, theygrind organic material and sediment into finer particles, mix up the substrate and recycle the detrital material on the top layers of the sediment which allow the penetration of oxygen in lagoons, reefs and other habitats (Bruckner *et al.*, 2003).Therefore, bottom feeder sea cucumber inhibits anaerobic process coupling with sulfide production in sediment (Kitano *et al.*, 2003) and promotes the increase of species diversity in the habitat.

Sediment is an absolutely necessary material for not only feeding of sea cucumbers but also for burrowing and wellbeing of these benthic organisms. Robinson *et al.* (2013) suggested that the presence of sand improves the growth due to the increased surface area for the development of natural food as bacteria and microphytobenthic primary production. Also, diet digestion facilitates by the presence of sand for consumption (Watanabe *et al.*, 2012). The presence of sediment reduces the stress impacts as limited growth rate caused by the handling and fluctuation in environmental parameters by allowing sea cucumbers to bury in accordance with their diurnal cycle and in response to stress conditions (Mercier *et al.*, 1999; Purcell, 2010; Robinson *et al.*, 2013).

Sea cucumbers can freely select sediment type and grain sizes among different locations in the wild by slow and steady movements. Although, it seems clear that an active choice occurs during the settlement of holothuroids, there is little knowledge on the processes determining their settlement (Chia and Walker, 1991; Mercier *et al.*, 2000; Smiley *et al.*, 1991).There are limited reports focusing on the sediment selectivity of sea cucumbers in the literature (Mercier *et al.*, 1999; Mezzali and Soualili, 2013; Slater and Jeffs, 2010; Uthicke and Karez, 1999), but none of them was concerning on the effects of sediment grain size on growth performance of sea cucumbers. Current extensive and intensive methods for sea cucumbers grow-out, including sea ranching, pen farming and pond culture, all require sandy-muddy sediment substrates (Duy, 2012; Hair, 2012; Juinio-Meñez *et al.*, 2012; Robinson and Pascal, 2012). As the sea cucumbers under aquaculture conditions have no choice for sediment type other than provided, the main difficulty associated with tank-based sea cucumber culture is the maintenance of optimum substrate conditions (Robinson *et al.*, 2013) .It is important to know the impact of sediment typeon feeding, burrowing and growth performance of sea cucumbers in capture conditions as in aquaculture.

The purpose of present study is to determine the proper sediment grain size which is commercially available on the market or naturally found in the habitat for optimal growth of *H. tubulosa* under aquaculture conditions.

Materials and Methods

Sample Collection

H. tubulosa juveniles were collected by scuba diving from 1-2 m depth in the *Posidonia oceanica* meadow and *Cystoseria barbata* macroalgae populated zones of east Aegean coasts (38°21'50.6"N 26°46'52.2"E and 38°38'30.5"N 26°31'35.5"E) between July and October 2012. Collected juveniles of similar sizes (30-40 g) were transferred to Ege University, Faculty of Fisheries, H.Okan Kamacı Aquaculture Research Unit in Urla, İzmir, Turkey within two hours. Sea cucumbers were placed into 2x2x1 m square polyester tank with sediment taken from habitat of the collected sea cucumbers. Sea cucumber juveniles were kept for 4 weeks for acclimatization. The salinity of seawater in the open circuit marine water tank was 39‰ and the temperature was 24°C as collection site of sea cucumbers. *H. tubulosa* juveniles were fed on dried and powdered macro algae (*Cystoseira* sp.) on daily basis. In the end of acclimatization period, specimen were transferred to bare aquaria and kept for 48 hours without any feed in order to enable them to release gut contents as indicated by Battaglene *et al.* (1999) prior to weighing.

Experimental Design

Sediment studies were carried out at the indoor aquaculture laboratories of Ege University Faculty of Fisheries for 61 days between October and December 2012. The experimental unit consisted of glass aquaria (200x50x50 cm) filled with 3 different grain sized SIO_3 quartz sediment and natural sand (control) by 10 cm height. Natural crushed quartz sediment was rinsed with fresh water and dried at 105ºC for 24 hours prior to the experiment in order to eliminate any substances and organic matter inside. The treatments were set in triplicate under three different sediment grain size diameters, 1 mm, 3 mm and 7 mm, named as A, B and C, respectively (Figure 1). Control tanks were filled with sandy-muddy sediment (0.05-0.2 mm) which was taken from the collection site of the sea cucumbers.

One third of the sea water in the treatment tanks were replaced every two days with pre-heated and filtered sea water. Additionally, treatment tanks were supplied with extra mechanic filtration by external filters at a rate of 150 L h^{-1} in order to maintain best water quality during the study.

The physico-chemical parameters of the seawater were kept similar among the experiment units. During the 61-day trial pH values did not vary between the groups and the average pH for all groups was recorded as 8.55 (min pH 7.55 - max pH 9.00). All experiment aquaria were heated by in-water heaters (Atman, 500 W) and water temperature was between 22.26ºC and 25.02ºC, as average 23.84±0.64ºC. The salinity level was average 36.87±1.93‰ (Atago Smill Refractometer) and there was no significant difference among the treatment groups. Dissolved oxygen level was measured by digital oxygen-meter (Hanna HI 9142) and kept between 7.2 mg L^{-1} and 8.5 mg L^{-1}, as average 8.03±0.39 mg.l^{-1}.The tanks were illuminated by 12h light: 12 h dark cycle with 4x40 W daylight type fluorescent lamps (Philips) settled 2 m above the water surface.

Average 35.02±0.52 g (min: 30 – max: 39.4 g) individuals were selected randomly from the acclimation tank and placed into experiment units by

350 g m^{-2} (10 juveniles m^{-2}) stocking density. Juveniles were fed with the formulated diet containing 85% fine granulated dry macroalgae *Cystoseira barbata*and 15% commercial fish feed by daily ration equal to 10% of wet biomass per tank (Table 1). The daily amount of feed was separated into two parts and given 2 times a day at 10:00 and 16:00. The growing microalgae on all sides of aquaria were cleaned and removed regularly. Excess feed and faeces were siphoned from the bottom of the tanks every morning.

Data Collection

Weight measurement is difficult in holothurians due to the variable amount of water in the respiratory trees, as well as ingested sediment in the gut (Seeruttun, 2008). In order to eliminate such measurement errors, juveniles were kept in bare aquaria without feed for 48 h prior to initial, mid and final weighing. *H. tubulosa* juveniles were then dried on a sponge to remove external water on their body surface and weighed within 1 minute by digital scale (OHAUS, Model: Scout Pro SPU 4001) as indicated by Battaglene *et al.* (1999) and Dong *et al.* (2006).

Specific Growth Rate (SGR) and Survival Rate (SR) was calculated to predict the effect of sediment grain size on growth performance of *H. tubulosa* juveniles among the treatment groups as stated below (Dong *et al.*, 2006).

Specific Growth Rate = $[\ln (W_2) - \ln (W_1) / t] \times 100$ (SGR - % day^{-1})

Survival Rate (SR - %) = $[N_f / N_i] \times 100$

where W_1 is mean initial wet weight, W_2 is mean final wet weight of sea cucumbers, t is the period of trial, N_f is number of sea cucumbers at the end of trial and N_i is the number of sea cucumbers at the start of trial.

Statistical Analysis

Normality of the data was determined by Kolmogorov–Smirnov test and homogeneity of the means was analyzed by Levene's test. All data were pooled prior to the analysis. Student *t*-test were used to compare growth data between treatment groups. The means were compared by one-way analysis of variance (ANOVA) and Tukey test was used for post hoc comparison of the means where significant. Data were presented as mean ±SD where means were considered significantly different at $P<0.05$. All statistical analyses were performed by SPSS 15.0 software.

Results

Final mean weight of the juveniles in sediment groups 1 mm, 3 mm, 7 mm and 0.2 mm (control) were recorded as 49.18±0.94 g, 44.23±0.35 g, 40.72±0.53 g and 39.57±0.50 g, respectively (Table 2). The mean weight decreases as the grain size increases except control group. As the artificial airflow and water circulation in limited area force tiny sediment particles to be moved continuously, the control group aquarium became turbid from the first week of the treatment.

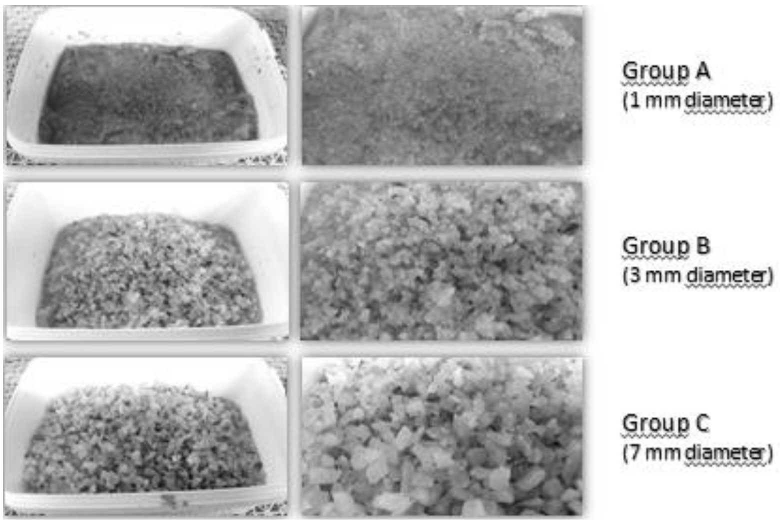

Figure 1. The sediment size and treatment group distribution.

Table 1. Proximate composition (%, wet wt.) of feeds used in diet

	Crude Protein	Crude lipid	Carbohydrate	Ash	Dry matter	Na Alginate
Cystoseirabarbata	5.57	0.01	63.97	15	90.77	17.30
Sea bass growing feed	45	20	-	11	90	-

Table 2. Growth performance of sea cucumber juveniles (*H. tubulosa*) on different grain sized sediments after 61 days

	Sediment groups			
	A (1 mm)	B (3 mm)	C (7 mm)	Control
Initial Weight (g)	35.00±0.29	35.07±0.27	35.00±0.28	35.10±0.34
Final Weight (g)	$49.18{\pm}0.94^{a}$	$44.23{\pm}0.35^{a}$	$40.72{\pm}0.53^{b}$	$39.57{\pm}0.50^{b}$
SGR (% day^{-1})	$0.53{\pm}0.03^{a}$	$0.38{\pm}0.02^{b}$	$0.24{\pm}0.03^{b}$	$0.20{\pm}0.02^{b}$
WG (g ind^{-1})	$14.18{\pm}0.58^{a}$	$9.16{\pm}0.43^{b}$	$5.72{\pm}0.27^{c}$	$4.47{\pm}0.21^{c}$
SR (%)	100±0.00	100±0.00	100±0.00	100±0.00

Superscripts indicate significant differences between sediment groups A, B, C and Control.
SGR=Specific growth rate; WG=Weight gain; SR= Survival rate.

The lowest mean final weight was recorded in 7 mm sediment group which is significantly different from the others ($P<0.05$). The individuals in 1 mm sediment group gained average 14.18±0.58 g ind^{-1} which is 0.23±0.03 g d^{-1}. The average weight gain in 3 mm sediment group was recorded as 9.16±0.43 g ind^{-1} and 0.15±0.01 g d^{-1}. The lowest average weight gain data recorded in 7 mm sediment and control group which were 5.72±0.27 g ind^{-1} and 4.47±0.21 g ind^{-1}, respectively. Final weight of 1mm and 3mm sediment groups significantly differed from the 7mm sediment and control groups ($P<0.05$). The average weight gain in 1 mm, 3 mm and 7 mm sediment groups found as significantly different among themselves ($P<0.05$). The 7 mm and control groups gained no significantly different average weight in 61 days ($P>0.05$).

Specific growth rate ranged from 0.20±0.02% day^{-1} (Control) to 0.53±0.03% day^{-1} (1mm). There was no significant difference between 3mm,7mm and control group in terms of specific growth rate ($P>0.05$). The highest specific growth rate as 0.53±0.03% day^{-1} recorded in 1mm sediment group was significantly different than the others ($P<0.05$). Overall, best growth performance was recorded in 1 mm sediment group.

No mortalities were observed during the experiment period and the survival rate was calculated as 100% for all the treatments.

Discussion

Hudson *et al.* (2005), reported that rich pigmented plankton communities and feed piles on the tiny sediment were the primary preferred zones in laboratory conditions for two shallow fjordic sea cucumber species. Moreover, Plotieau *et al.* (2013) suggested that the nutritional value of the fine sediments would be higher than that of the coarse sediments because of their higher number of nutritive microorganisms. In agreement with the findings of Hudson *et al.* (2005) and Plotieau *et al.* (2013), *H. tubulosa* juveniles in this study were cumulated on the mass plankton and detritus piles which were easily noticeable on the small grain sized sediments.

The current study revealed that *H. tubulosa* could benefit from 1mm to 3 mm grain sizes in order to get best growth performance, where the growth rate negatively affected by increase in grain size. These findings are parallel with Mezzali and Soualili (2013)'s study that reports *H. tubulosa'*s significant settlement and feeding preference for the average grain size between 0.2 and 2 mm.

The coarse sediment in this study with 7 mm grain size is bigger than the average 5 mm mouth opening of juvenile sea cucumbers, therefore *H. tubulosa* juveniles did not prefer to take this sediment into their mouth and cannot be able to pass through their guts. The sea cucumber juveniles in 7 mm group had probably consumed only the limited detritus and cumulated feed in the spaces among grains without utilizing from the algae and the other microorganisms that were developed on the sediment grains. Watanabe *et al.* (2012) suggested that diet digestion was facilitated by the presence of sand available for consumption and Berthois *et al.* (1968) suggested that the rate of organic matter increases with the importance of the fine fraction of the sediment. Sand may be an essential part of sea cucumber diets but the size limitation for 7 mm group in this study could negatively effected the growth of these sediment feeders.

Pitt *et al.* (2001) reported holothurians prior preference for sandy and sandy-muddy substrates, however current observations revealed that such sediments are not suitable in aquaculture tanks where the optimum culture conditions are maintained by outer interventions. The interventions as aeration and water changes had forced tiny and muddy sediment of control group to be mixed frequently in large amounts. This movement cause sediment, detritus and bacteria to mix continuously in the water column rather than to be accumulate on the upper sediment layer. As *H. tubulosa* is not a suspension feeder, the poor nutrient content of sediment would cause the lowest average weights and growth rate in this group. Although, sandy and muddy sediment is the natural habitat of *H. tubulosa*, such sediment type is not suitable for tank based aquaculture with strong aeration and water flow. Besides, coarse sediment which cannot move with the hydro dynamism could be more convenient to the feeding behavior of sea cucumbers. The findings in this study is parallel with the results of Robinson and Pascal (2012) who suggested that sandy-muddy sediment substrates are essential in extensive aquaculture methods including sea ranching, pen farming and pond culture but maintaining optimum substrate conditions is not easy in tank-based sea cucumber culture with such sediment. In addition, the limited growth performance of *H. tubulosa* under suspended sediment and muddy water conditions has been revealed by this study.

This research is the unique reference in literature about the effect of sediment grain size on growth of sea cucumber *H. tubulosa* under tank-based aquaculture conditions. The future researches with different sediment types in various conditions and in latter development phases of sea cucumbers would be beneficial for the efficient production of *H. tubulosa* for aquaculture.

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