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Sea cucumber (*Holothuria tubulosa* Gmelin, 1790) culture under marine fish net cages for potential use in integrated multi-trophic aquaculture (IMTA)

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In this study, integrated aquaculture of Mediterranean sea cucumber species *Holothuria tubulosa* under finfish net cages was investigated. To evaluate whether *H.tubulosa* can be reared under net cage farms without additional feed and to compare growth performances, three underwater ranches (10x10m), just below a net cage (A), between the net cages (B) and 750 m away from the net cage systems (K) were set. Fifty sea cucumbers (92.81±2.29 g) were placed into each research station and growth performances like specific growth rate (SGR) and weight gain were observed for 90 days. SGR of sea cucumbers in stations A, B and K were 0.32±0.04, 0.22±0.02 and 0.10±0.01 %/d, respectively. The highest mean weight gain 31.11±0.84 g was recorded at station A where the lowest was in station K as 8.89±0.12 g. Organic carbon and organic material amount of research stations significantly affected the final mean weights of sea cucumbers where the highest growth was determined at the stations A and B. The results suggest that integrated multi-trophic aquaculture (IMTA) systems combined with finfish and sea cucumber *H. tubulosa* under net cages would provide by-products to aquaculture farms without an extra feed costs in the Mediterranean Sea.

[**Keywords:** Biodeposit, mariculture, marine ecology, Mediterranean Sea, polyculture]

Introduction

Global supply of fish has reached to 163 million tons in 2013 which aquaculture contributed 43.1 percent of this production. Aquaculture production was reported as 70.2 million tons that nearly 25.5 million coming from the mariculture. While the production from capture fisheries has exceeded the sustainable limits of wild stocks, aquaculture currently provides nearly half of the fish consumed worldwide[1]. In terms of fish production, future predicts agreed on that aquaculture will contribute 62 percent of the global supply by 2030[2]. Aquaculture is a good opportunity for supplying fish to the consumer through the whole year and reducing pressure on wild fish stocks as well as a huge economic subsector by nearly 150.3 million USD production value[1]. On the other hand, aquaculture production may severely degrade aquatic ecosystems[3]. The most common environmental problems include pollution of aquatic and benthic ecosystems[4]. Many researches on the ecological impacts of marine aquaculture systems suggest a significant increase in eutrophication and sedimentation[5,6] within a radius about 100 m around the net cage systems[7,8]. This effect on the environment mainly caused by the organic output from net cages composed by excess feed and fish faeces[5,9]. Phosphorus and nitrogen rich biodeposits sink rapidly to the seafloor and mainly accumulate under the net cages from 8 to 25 times more than the locations 1 km away from the net cages[10]. Such biodeposits alter sediment[11] and water column characteristics which negatively affect benthic ecosystem close to the net cages and immune system of the fish inside the net cages. The latter effect may impress the technical and economic productivity of marine net cage farms due to the increasing mortality.

Despite all these negative impacts, such nutritional waste would be a valuable diet for some other economic species. Integrated multi-trophic aquaculture (IMTA) is a considerably new method widely used in natural transformation of biological wastes (uneaten feed and faeces of fish) to nutritional feed for lower trophic level extractive species[12,13]. An aquaculture farm can take advantage of IMTA by harvesting commercially valuable organisms (seaweeds, molluscs and sea cucumbers)[14] those fed on primary production's nutritional waste. Furthermore, IMTA provides environmental sustainability through biomitigation and economic stability through product diversification and risk reduction[12].

Sea cucumbers are the best components of IMTA as they are bottom dwellers which called worms of the sea[15]. Sea cucumbers, the members of Holothurian family, are sediment filtering organisms that assimilate live diatoms, bacteria, detritus and low content organic material by passing a large amount of sediment through their guts[16]. This process provides bioturbation inside the sediment[17].Sea cucumbers are harvested and traded in more than 70 countries worldwide[18] where more than several hundred species are currently exploited commonly in various regions of the World[19].

IMTA operations which include sea cucumbers and other species turn aquaculture waste into a marketable product[20]. James, et al. [21] reported that polyculture of shrimps and sea cucumbers (*Holothuria scabra,* Jaeger 1833) in the soil ponds had improved the bottom characteristics, reduced the organic matter quantity and increased the growth rate of the shrimps. Similarly, Chen [22] reported good survival rates for sea cucumbers (*H. scabra*) co-cultivated with shrimps (*Penaeus monodon,* Fabricius 1798) and sea snails (*Babylonoia aerolata,* Link 1807). Ito [23] reported a better growth for co-cultured *Apostichopus japonicus* (Selenka, 1867) juveniles with sea urchins than monocultured juveniles. Slater, et al. [24] reported that *Australostichopus mollis* (Hutton, 1872) beneath mussel farms significantly decreased the level of organic carbon also *A. mollis* juveniles fed on the mussel waste diet exhibited the greatest specific growth rate (SGR) and overall growth performance. Aquaculture farms would also benefit from the reclamation of the environment by deposit feeding sea cucumbers in an integrated production.

Although the successful culture of sea cucumbers in IMTA has been reported in all these studies, none of these species are habitants of the Mediterranean Sea where 20 976 marine cage culture farms[25] producing 199 542 tons of marine finfish per year. Moreover, there is no report in the literature about inclusion of Mediterranean sea cucumbers in such IMTA systems, whereas 47 species of sea cucumbers found in the Mediterranean Sea[26] which the commercial species[27] are *Holothuria tubulosa* (Gmelin,1790), *Holothuria sanctori* (Delle Chiaje, 1823), *Holothuria poli* (Delle Chiaje, 1824), *Holothuria mammata* (Grube, 1840), *Stichopus regalis* (Cuvier, 1817) and *Holothuria forskalii* (Delle Chiaje, 1823). There are also successful studies on preliminary aquaculture of *H. tubulosa*[27,28] and *H.forskalii*[29] but net cage aquaculture farms did not benefit from such Mediterranean species in IMTA systems since now.

The aim of this research is to reveal the feasibility of sea cucumber *H. tubulosa* breeding under the finfish net cages in integrated multi-trophic aquaculture systems as a candidate product for Mediterranean aquaculture which would increase the profitability of the farms and welfare of the fish inside the net cages.

Materials and Methods

Experiments aimed to evaluate growth performance of Mediterranean sea cucumber H. tubulosa, in IMTA systems under the fish net cages were conducted at a marine fish farm (38.2205694° N, 026.4281306° E) in Mersin Bay, Izmir-Turkey (Fig. 1). The fish farm was producing European seabass (*Dicentrarchus labrax*, Linnaeus, 1758) and gilthead sea bream (*Sparus aurata*, Linnaeus,1758) in 10 m diameter and 10 m deep circular net cages with a capacity of 200 tons per year.

Fig. 1— Research location

The selection of research stations was based on the influence rate of net cage plants on the organic carbon content (C%) of the sediments[8]. Sediment samples were collected by scuba-divers from various locations around the net cages and analysed for organic carbon content according to Gaudette et al.[30]. The first station named as (A) was located just below the net cage plant which has 12 circular net cages. The second station (B) was located in the middle of two net cage plants and approximately 70 m away from both plants. The control station (K) was located approximately 750 m away from all net cage plants and 20 m close to the coastline (Fig. 2). All stations were on the seafloor where the depths of water column for A, B and K were 20 m, 18 m and 4 m, respectively.

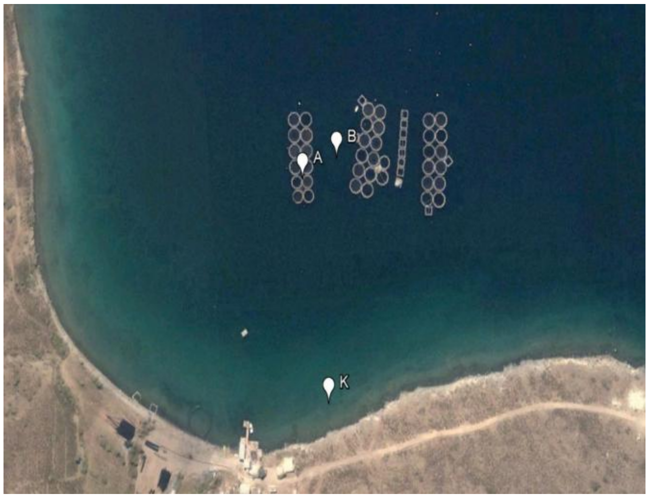

Fig.2—Research stations located around the net cages

The research stations were set as 10 x 10 m square areas surrounded by 40 x 2 m polyamide fish nets with 22 mm mesh size (Fig. 3). The fish nets were hanged on 13 pieces of 2 m long wooden strikes for each station to form an underwater fence. Wooden strikes, painted with antifouling paint to increase the endurance, stuck to the sea bottom around the research stations. Polyester buoys were attached to the top line of fish nets to maintain buoyancy and bottom line of nets were buried about 50 cm under the sea floor and fixed by stones, chains and strings.

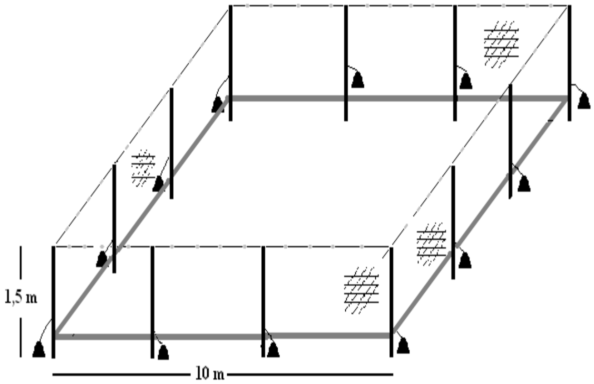

Fig. 3—The shape of research stations

Prior to the experiment, faunal biodiversity and presence of *H.tubulosa* in the research zone were verified by several qualitative observations of scuba-divers. *H.tubulosa* individuals were collected from the Mersin Bay and kept in bare polyester tanks under running sea water for 24 h in order to evacuate sediment in their guts. After the starvation period, *H. tubulosa* individuals were weighed on digital scale (OHAUS, Model: Scout Pro SPU 4001) as indicated by Battaglene et al.[31] and 50 sea cucumbers ranging between 90 to 95 g (92.81±2.29 g) were placed in each research station in triplicated treatments. In order to ensure continuous feeding of sea cucumbers throughout the experiment, the period was selected between July and October for 90 days.

Water samples were taken from bottom water approximately 50 cm above the sediments by using a Niskin bottle bi-weekly for determination of physical and chemical conditions and organic material. The sediment samples were taken from the production stations bi-weekly for inspection of organic carbon (C%). Water and sediment samples were brought to the laboratory and analysed within a few hours. Dissolved oxygen and pH were measured by digital oxygenmeter (WTW Oxi 330) and pHmeter (OAKTON). The water temperature was measured by SCUBA divers' digital thermometer (MARES Puck Pro) in each sampling zone. Salinity was measured by Mohr Knudsen titration method[32] and organic material (OM) was inspected by $KMn0_4$ titration[33]. Organic carbon (C%) in sediment samples were determined by Modified Walkley-Black titration method[30].

The wet weights of sea cucumbers in each production station were measured every 30 days. Sea cucumbers were collected from the research stations by SCUBA divers, transferred to the coast in water circulated polyester tanks, weighed on digital scale according to Battaglene et al.[31] and returned to the stations where they came from. All individuals were kept in bare polyester tanks under running sea water for 24 h before the final weighing. Weight gain and SGR of sea cucumbers used as growth performance indicator. Weight gain (WG) and specific growth rate (SGR) were calculated by the formula:

$$\text{WG (g)} = W_t - W_i$$
$$\text{SGR (\%/d)} = 100[\ln(W_t) - \ln(W_i) / t]$$

where W_t and W_i are final and initial wet weight of sea cucumbers in each station (g); t = duration of experiment (days).

Statistical analyses

Normality and homogeneity of the obtained data from production stations were tested by Shapiro-Wilk and Levene's test, respectively. One-way ANOVA was used to determine the differences between initial and final weights of the sea cucumbers. The significant differences between mean weights of triplicated treatment groups were identified by pairwise comparison using Tukey's tests. In case of non-parametric data, Kruskall-Wallis test was used to determine the significance among variables. Student t-test was used to examine the differences in water parameters and sediment analysis. All data was given as mean±standard deviation (SD) and the differences were considered as significant if P<0.05. Statistical analysis software SPSS v.15 was used to perform all statistical analysis.

Results

Water temperature in production stations A and B at 20 m depth were not changed significantly throughout the research period and recorded as mean values of 19.59±0.80 ^{0}C and 20.17±1.34 ^{0}C, respectively. Water temperature of station K in shallow zone (4 m) was slightly higher, but not significantly different than the other stations (P>0.05). Seawater salinity was not changed significantly during the research period and was not significantly different among the research stations (P>0.05). The mean salinity was recorded approximately 37.5 ppt for all stations. pH was 7.25-7.73 and not significantly different between three research stations (P>0.05). Dissolved oxygen at all research stations was >6 mg/l (Table 1).

Initial mean wet weight of sea cucumbers at the station A was 93.78±6.11 g and final mean wet weight was recorded as 124.89±4.75 g at the end of 90 days. Highest mean weight gain was recorded at station A as 31.11±0.84 g. Mean weight gain at station B was recorded as 20.35±0.61 g which is significantly different than station A and station K (P<0.05). Initial mean weight at station B was 92.67±5.06 g while final mean weight was recorded as 113.02±6.78 g. The lowest weight gain was recorded at station K as 8.89±0.12 g which the initial mean wet weight was 92.00±4.70 g and final was 100.89±5.75 g (Table 2). The weight difference for the sea cucumbers was found as significant between all stations (P<0.05) (Fig.4).

Growth performance as SGR of sea cucumbers at station K was significantly lower than stations A and B (P<0.05). The SGR of sea cucumbers at stations A, B and K were recorded as 0.32±0.04, 0.22±0.02 and 0.10±0.01 %/d, respectively (Fig. 5).

Table 1—Physical and chemical conditions of research stations during the research period

Stations	Temperature (°C)	Salinity (ppt)	pH	Dissolved oxygen (ppm)
A	19.5 ± 0.80^a	37.92 ± 0.67^a	7.73 ± 0.70^a	6.44 ± 0.87^a
B	20.17 ± 1.34^a	37.33 ± 0.78^a	7.68 ± 0.70^a	6.33 ± 0.55^a
K	21.56 ± 1.21^a	37.59 ± 0.54^a	7.25 ± 0.90^a	6.73 ± 0,46^a

Values are given as means ± SD. Different subscribed letters indicate significant differences between research station

Table 2—The mean wet weight (g) of sea cucumbers in research stations

Stations	Day 1	Day 30	Day 60	Day 90
A	93.78±6.11^a	102.41±3.54^a	114.67±5.31^a	124.89±4.75^a
B	92.67±5.06^a	96.85±2.95^b	100.89±3.85^b	113.02±6.78^b
K	92.00±4.70^a	95.23 ± 2.72^b	97.11 ± 3.41^b	100.89±5.75^c

Values are given as means ± SD. Different subscribed letters indicate significant differences between research stations.

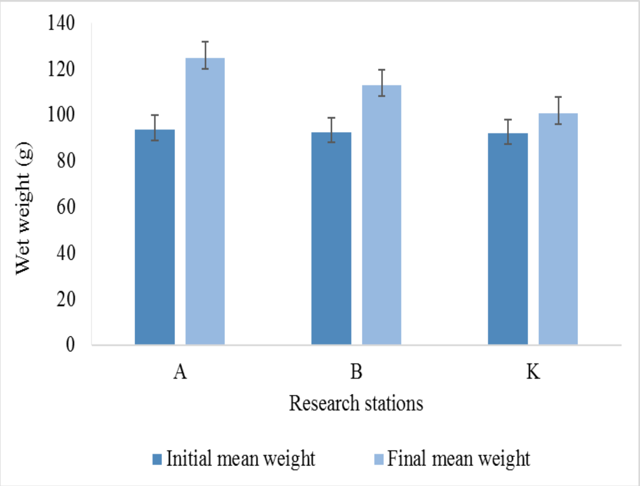

Fig. 4— Initial and final weight difference between research stations.

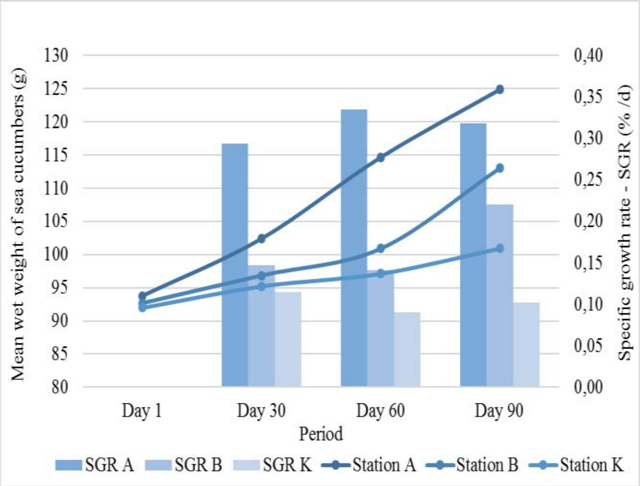

Fig. 5— Specific growth rate and mean wet weight of sea cucumbers

Organic carbon content (C%) of the sediment differed significantly between control (K) and research stations (A and B) under the net cages ($P<0.05$). Organic carbon percent in the sediment of control station which is 750 m away from the net cages was recorded as 0.10-0.16 % and not changed significantly till the end of research period ($P>0.05$). In August and September, organic carbon percent of sediments at stations A and B slightly higher than July, but not changed significantly ($P>0.05$).

Organic material in water column just above the sediment of research stations was nearly doubled for all stations between months July and August and slightly decreased at month September. Organic material content in water column above the control station (K) was significantly lower than other research stations ($P<0.05$). The mean organic material amount in month August differed significantly for all research stations ($P<0.05$) (Table 3).

Table 3—Organic carbon (%) and organic material (mgKMnO4 lt-1) variation at research stations (A: under cage; B: between cages; K: control) during research period

	OC (%)			OM (mgKMnO$_4$ lt^{-1})		
	July	August	September	July	August	September
A	4.68±0.28^a	4.84±0.62^a	4.32±0.51^a	60.04±5.06^a	156.74±11.89^a	129.56±10.72^a
B	3.64±0.21^a	4.20±0.46^a	3.89±0.30^a	53.72±4.97^a	129.39±10.71^b	116.83±9.87^a
K	0.16±0.07^b	0.13±0.04^b	0.10±0.03^b	24.83±2.23^b	53.56±4.63^c	33.24±3.41^b

Values are given as means ± SD. Different subscribed letters indicate significant differences between research stations

Organic carbon and organic material amount of research stations significantly affected the final mean weights of sea cucumbers ($P<0.05$). The highest growth was determined at the stations A and B which have high organic carbon and organic material while the lowest growth recorded at station K that has the lowest organic carbon and organic material (Figs.6 and 7).

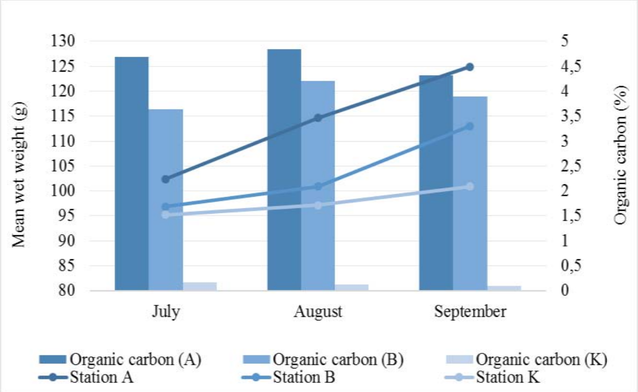

Fig. 6— Organic carbon and mean wet weight of sea cucumbers during the research period

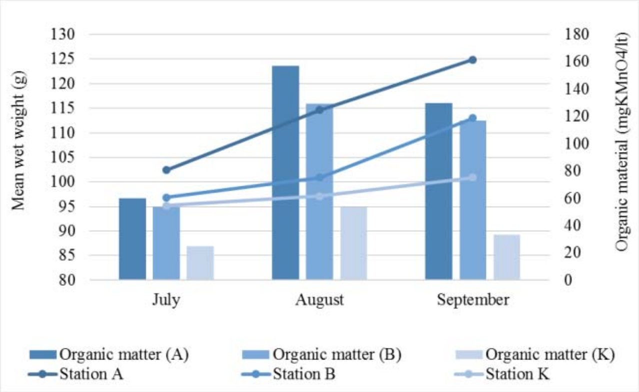

Fig. 7— Organic material and mean wet weight of sea cucumbers during the research period

Discussion

The negative impacts of marine aquaculture wastes as faecal matter, waste food and metabolic products on organic carbon and nitrogen composition of sediments below and around the production systems were documented by several studies[6,9,11]. Moreover, Grenz et al.[34] noted that such organic carbon and nitrogen content of sediments are increasing over time with continuous deposition due to the growth of bacterial biomass. Similarly, the organic carbon and organic material amount below the net cage systems found as significantly higher than the control station which is approximately 750 m away from the net cages in this study. The organic carbon percent in the sediment of control station was within the range from 0.10±0.03to 0.16±0.07 % which is similar to the results of Aloupi and Angelidis[35] who reported 0.11 to 0.93 % organic carbon in the coastal sediments of Lesvos Island in the Aegean Sea.

Isgoren-Emiroglu and Gunay[36], reported that such organically rich sediment below the net cages forms a valuable feed source for deposit feeders as sea cucumbers. Conforming the suggestions of Isgoren-Emiroglu and Gunay[36], Yokoyama[20] found a considerable SGR as 1.3 %/d for the sea cucumber *A. japonicus* reared below a fish cage. In the present study, *H. tubulosa* under net cage systems grew better than the ones in control station during 90 days. Weight gain and SGR at station A which is just below the net cage, found as 31.11±0.84 g and 0.32±0.04 %/d, respectively. Although, such SGR was comparable with that of other sea cucumber species co-cultured with red sea bream[20], it was within the range from 0.20 to 0.53 %/d for *H. tubulosa* cultured at tank conditions[28] and similar with the SGR (0.27-0.30 %/d) reported by Zhou, et al.[37] for *A. japonicus* co-cultured by bivalves. SGR of station B was lower than station A but it was still within the range of tank reared *H. tubulosa* while the SGR of control station (K) was significantly lower than other stations. Such difference would be a result of excessive organic carbon and organic material accumulation under net cage systems so that organic carbon and organic material content of station K were also significantly lower than the others. Similarly, Ahlgren [38] suggested that the net cage systems supply excessive food environment for sea cucumbers. Furthermore, previous studies on feeding selectivity of various sea cucumber species revealed that high organic nutrient sites are preferable by such deposit feeders[16,39] and better growth was obtained on organically rich sediments[29,36].

In this study the sea cucumber production stations at 18-20m depths were within the natural habitats of *H. tubulosa*[40], however, our study revealed that harvesting of sea cucumbers in open net farms under net cages require excessive labour via scuba diving and handpicking of sea cucumbers at such depths. Moreover, open net farms are easily subject to predator attacks and sea cucumbers can easily escape from the production area by climbing up or crumbling under the net walls. The best solution for those problems would be modular box cages for sea cucumber production that lay on sea floor below the net cage systems that are easily transferable, keep sea cucumbers in safe and allow accumulation of biodeposits inside.

Conclusion

Integrated multi-trophic aquaculture is a considerably new economic and ecological production system which combines the production of species from different nutritional levels in the same system. In this respect, this study revealed that IMTA systems combined with aquaculture species and sea cucumber *H. tubulosa* in the Mediterranean Sea would provide by-products to net cage farms without extra feed costs. This study investigated co-cultivation of sea cucumber *H. tubulosa* under the marine net cage systems in the Mediterranean Sea for the first time but future studies on other economic sea cucumber species and production systems under net cages would be required.

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