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PRELIMINARY STUDY OF SETTLEMENT OF FLAT OYSTER SPAT (*OSTREA EDULIS* L.) ON OYSTER AND MUSSEL SHELL COLLECTORS

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Key words: mussel shells, *Ostrea edulis*, oyster shells, spat recruitment

Abstract

Oyster recruitment was monitored in Karantina Island near Izmir, Turkey, from May to October 1999. The aim of the study was to determine the recruitment pattern for six months and the most suitable time to collect oyster spat from nature. Oyster and mussel shells were used as substrates in spat collectors. Five groups of each type of collector were deployed from May to August. The recruitment levels were highest in the groups deployed on June 16, with 4069 and 1575 oyster spats collected on the oyster and mussel shell collectors, respectively. Spat recruitment varied considerably between collector types as well as between the outsides and insides of shells (p<0.05). Spats larger than 10 mm were found on the second or third sampling date in each group for both oyster and mussel shell collectors. Barnacles (*Balanus* sp.) settled on both collector types in high numbers throughout the study. Data suggest that maximum recruitment in this area is most likely to occur on both collector types between May and mid-June.

Introduction

Larvae settlement behavior and choice of substratum for attachment are critical to the success of adult sessile marine invertebrates (Turner et al., 1994; Pillay, 1997). Substrates have been used to enhance oyster settlement since Roman times (Plinius in Mann et al., 1990). Various materials have been examined including mangrove roots, bivalve shells, PVC, wood, fiberglass, car tires, and tiles (Angell, 1972; González, 1976; Wedler, 1980; Narciso, 1995; Holiday, 1996; Buitrago and Alvarado, 2005). Empty oyster and mussel shells are extensively used in culture operations to collect oyster and mussel spat (Ogle and Chestnut, 1979; Heral et al., 1989).

Because of natural fouling by sedimentation and organisms that compete for available settling space (Manning, 1952; Shaw, 1967;

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Abbe, 1988), the time selected for planting substrate collectors is critical to the successful recruitment of oyster spat. Shells planted too early in the year may become heavily fouled prior to the beginning of the settlement season. However, if shell clutches are planted too late in the season, the peak oyster settlement period could be missed (Morales-Alamo and Mann, 1990).

The breeding season of the flat oyster (*Ostrea edulis,* L.) in Izmir Bay, Turkey, where the majority of oyster beds are found, lasts from March to September, with a peak occurring from April to June (Yolkolu and Lok, 2000).

To our knowledge, no data on the recruitment of oyster spat in Turkey have been published. The primary objectives of this study were: (a) to determine the peak season for oyster spat attachment, (b) to compare the efficiency of oyster and mussel shells as collectors; (c) to observe the growth of settled oyster spat on both collectors; and (d) to identify the species recruited on the collectors.

Materials and Methods

Site description. The study site at Karantina Island is located at 38°22”44N, 26°47”12E on the west side of Izmir Bay (Fig. 1). The water depth is approximately 5 m at low water and the bottom is muddy sand.

Hydrographical features. Salinity (‰) was measured using a hand refractometer and temperature using a mercury-in-glass thermometer with a range of -10 to 100±0.5°C. Total particulate matter (TPM; seston) and chlorophyll *a* were measured by filtering with GF/C filters (Strickland and Parsons, 1972).

Experimental design. Empty oyster and mussel shells measuring 60-70 mm and 50-60 mm, respectively, were deployed on longlines (Karayücel et al., 2002) spaced one meter apart, approximately 70-80 cm below the water surface. Five groups of each type were installed: group I on May 3, group II on May 25, group III on June 16, group IV on July 11, and group V on August 1, 1999. Each group consisted of 12 collectors, to allow one for each of 12 potential samplings. The left

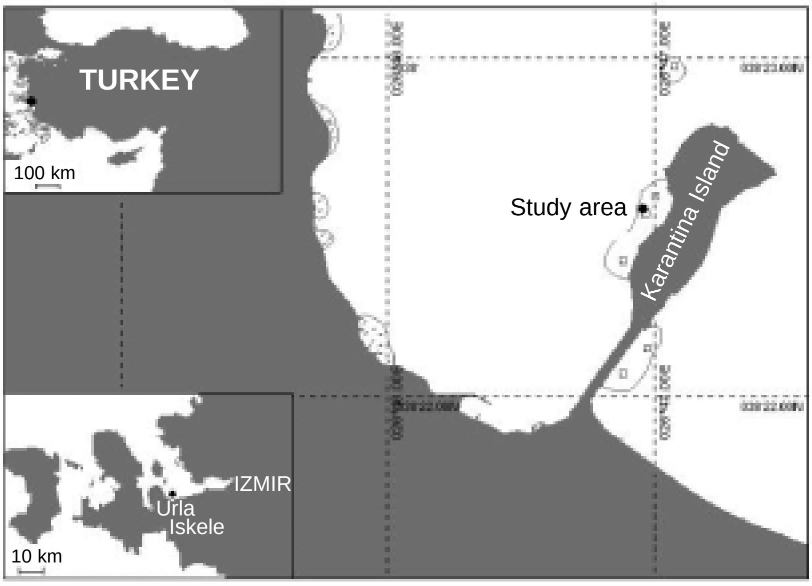

Fig. 1. Map of area in which oyster spat was collected.

sides of oyster shells were used; 30 were threaded together through holes driven in the centers of the shells (Fig. 2). Since mussel shells are thin and tend to break, they were placed into 12 mm mesh net bags (20 x 30 cm) at 30 per bag. Observations continued until no new spat attached, resulting in eight samplings. The last sample was taken on October 25 and the remaining collectors were ignored.

Sampling. One oyster and one mussel collector from each group was taken to the laboratory and visually observed. Attached spats were identified. The numbers of oyster spat on the inside and outside shell surfaces were counted and the lengths of the spat were measured from the umbo to the farthest point on the posterior edge of the shell. Colonization of fouling organisms such as barnacles, polychaete, and other bivalves was estimated. The abundance of each species and family was calculated and spat density was described as the number of spat per shell.

Statistical analysis. Significant differences between months and groups were analyzed by the Kruskal-Wallis test. Differences between settlements on the insides and outsides of shells were determined by the Mann-Whitney U test. Chi-square (χ^2) analysis was used to find differences between oyster and mussel shell collectors. Statistical tests were performed using SPSS 11.0 software. Differences were considered significant when $p<0.05$.

Results

Hydrographical features. Both temperature and salinity reached their maximums on July 11 (28.9°C and 37 ppt; Fig. 3). TPM ranged 12.9-36.5 mg/l and chlorophyll *a* 1.5-9.55 µg/l.

Spat settlement. The setting season lasted from May to October. Settlement began in June on oyster shell collectors and in May on mussel shell collectors (Fig. 4). There were obvious temporal and spatial differences in the number of spat found on the collector types. Recruitment on oyster shell collectors in group I peaked in the first week of July while recruitment on mussel shell collectors peaked in the second week of August. Spat settlement was significantly higher on the outer surfaces (65.8%) of oyster shells but more spat (60.4%) settled on the insides of mussel shells, except in Group I. The maximum total number of spat for both types of collector was obtained in Group II (Table 1). Total settlement was significantly higher on the oyster shell collectors (9238, 11.40 spat/shell) than on the mussel shell collectors (4225, 5.21 spat/shell). The mean number of spat over 10 mm increased with time in all groups except mussel shell collectors in groups III and V. Spats larger than 10 mm were found on the second or third sampling date. Spats larger than 20 mm were observed on the third or fourth sampling date for the oyster shell collectors and on the third sampling date for those groups of mussel shell collectors in which this size was observed.

Seven additional bivalve species were collected (Table 2). More *Mytilus galloprovincialis* settled on oyster shell collectors than mussel shell collectors. *Anomia ephippium* was the dominant fouling bivalve on both types, with more specimens on mussel shells than on oyster shells. Barnacles settled in high numbers throughout the study (Table 3). Polychaeta attached to oyster shell collectors beginning in August. A small number of bryozoans settled on the inside and outside surfaces of both types of shell. Few *Ascidiacea* were observed.

Discussion

Shell collectors deployed as early as mid-June or as late as early August can produce commercially acceptable recruitment of *Crassostrea virginica* in Maryland (Kennedy, 1980) and Jones Shore, Chesapeake Bay (Morales-Alamo and Mann, 1990), with annual variations in spatfall peaks.

The successful collection of wild mollusk spat for mariculture depends on a consistent supply of larvae with a high rate of settlement and subsequent survival (O'Beirn et al., 1996). The preferred settlement substrate for oyster larvae is a hard substratum, particularly oyster shells (Ulanowicz et al., 1980). Korringa (1976) reported that a satisfactory amount of spat (*Crassostrea gigas*) recruit-

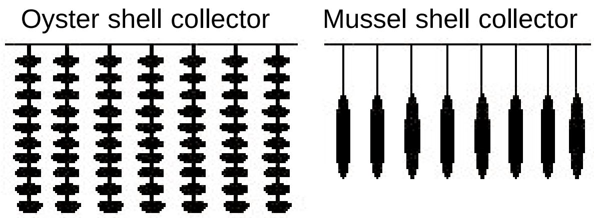

Fig. 2. Oyster and mussel shells hanging from long-lines to collect spat of the oyster, *Ostrea edulis*.

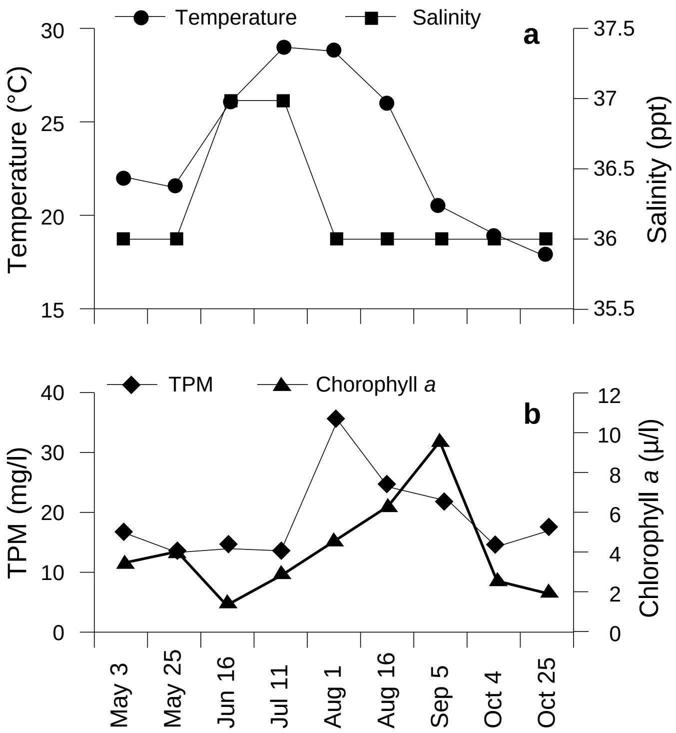

Fig. 3. Temperature and salinity (a), total particulate matter (TMP) and chlorophyll *a* (b) during the study period.

ment is 15-20 spat/scallop shell in Hiroshima Bay, Japan, and ≥30 spat/oyster shell in Willapa Bay, Washington. In comparison, we recruited 11.4 spat per oyster shell (means of pooled data) in group II and 5.21 spat per mussel shell. These differences seem to be related to the differences in surface area of oyster and mussel shells. Morales-Alamo and Mann (1990) obtained 1.8-2.3 *C. virginica* spat per shell in June in Chesapeake Bay while Adams et al. (1991) obtained 26.7 *C. virginica* spat per oyster shell on subtidal off-bottoms, 0.2 on subtidal bottoms, and 0.9 on intertidal bottoms. Lindsay et al. (1959) and MacKenzie (1981, 1996) suggest that 2 and 35 *C. virginica* spat per oyster shell constitute

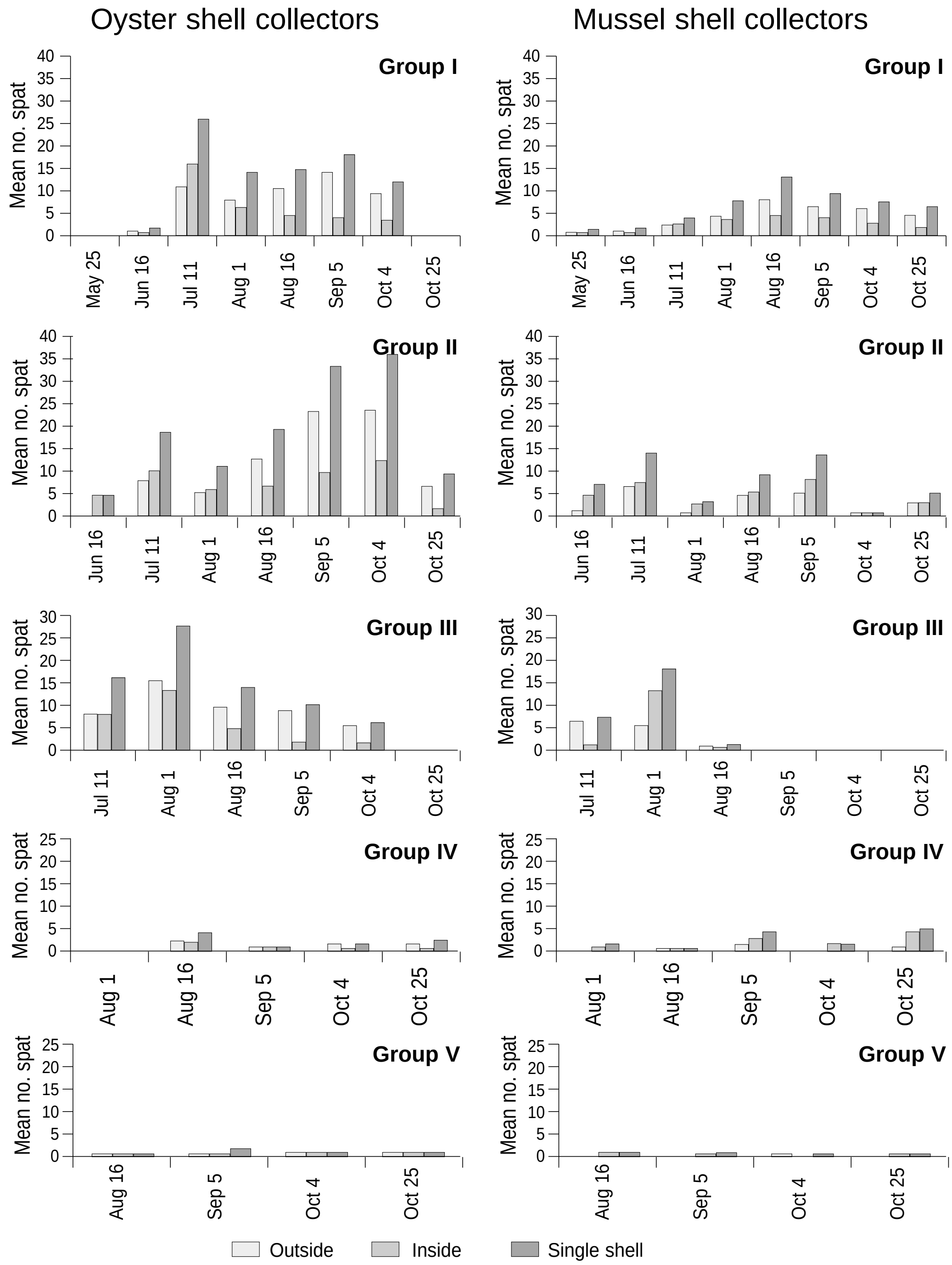

Fig. 4. Mean number of oyster spat settled on inside and outside surfaces of oyster and mussel shells used in collectors deployed on May 3 (group I), May 25 (group II), June 16 (group III), July 11 (group IV), and August 1 (group V) near Izmir, Turkey.

Table 1. Mean number and size distribution (%) of settled oyster, *Ostrea edulis*, spat on oyster and mussel shell collectors.

	Oyster shell collectors					Mussel shell collectors				
Sampling date	*Spat/collector*	*Spat/shell*	*<10 mm*	*10-20 mm*	*>20 mm*	*Spat/collector*	*Spat/shell*	*<10 mm*	*10-20 mm*	*>20 mm*
Group I (deployed May 3)										
May 25	-	-	-	-	-	20	0.66	100	-	-
June 16	42	1.4	92.8	7.2	-	39	1.3	88.8	11.2	-
July 11	786	26.2	95	5	-	114	3.8	50	-	50
Aug 1	416	13.86	81.25	6.25	12.5	220	7.33	50	21.42	28.57
Aug 16	438	15.08	50	50	-	367	12.23	43.7	31.25	25
Sep 5	528	17.6	-	87.5	12.5	278	9.26	73.3	26.6	-
Oct 4	357	11.9	-	26.3	73.7	228	7.6	12.5	81.25	6.25
Oct 25	-	-	Lost	Lost	Lost	177	5.9	32	68	-
Total	2567	10.69				1443	6.01			
Group II (deployed May 25)										
June 16	150	5.0	100	-	-	207	6.9	100	-	-
July 11	573	19.1	100	-	-	420	14.0	100	-	-
Aug 1	350	11.66	50	50	-	99	3.3	88.8	11.2	-
Aug 16	595	19.83	80	20	-	273	9.1	71.42	28.57	-
Sep 5	1020	34	-	93.75	6.25	402	13.4	78.57	21.42	-
Oct 4	1102	36.76	-	76.92	23.2	12	0.4	25	75	-
Oct 25	279	9.3	-	78.26	21.73	162	5.4	75	25	-
Total	4069	19.37				1575	7.5			
Group III (deployed June 16)										
July 11	483	16.1	100	-	-	210	7.0	100	-	-
Aug 1	840	28.0	100	-	-	544	18.13	100	-	-
Aug 16	417	13.9	40	48	12	21	0.7	100	-	-
Sep 5	309	10.3	66.6	25	8.3	3	0.1	100	-	-
Oct 4	183	6.1	8.3	50	41.6	-	-	-	-	-
Oct 25	6	0.2	50	50	-	-	-	-	-	-
Total	2238	12.43				778	4.32			
Group IV (deployed July 11)										
Aug 1	-	-	-	-	-	33	1.1	100	-	-
Aug 16	120	4.0	88.8	11.2	-	12	0.4	100	-	-
Sep 5	9	0.3	50	50	-	120	4.0	58.33	33.3	8.3
Oct 4	51	1.7	18.18	45.45	36.6	51	1.7	70	30	-
Oct 25	57	1.9	11.11	44.44	44.44	144	4.8	-	58.3	41.66
Total	237	1.58				360	14.4			
Group V (deployed August 1)										
Aug 16	13	0.43	100	-	-	24	0.8	100	-	-
Sep 5	63	2.1	100	-	-	21	0.7	100	-	-
Oct 4	36	1.2	14.28	85.71	-	7	0.23	100	-	-
Oct 25	15	0.5	75	25	-	17	0.56	100	-	-
Total	127	1.05				69	0.57			

Table 2. Abundance of *Mytilus galloprovincialis* (1), *Anomia ephippium* (2), *Anadara* sp. (3), *Chlamys* sp. (4), *Cardium* sp. (5), *Tapes* sp. (6), and *Modiolus* sp. (7) on oyster and mussel shell collectors.

	Oyster shell collectors						*Mussel shell collectors*						
Sampling date	*1*	*2*	*3*	*5*	*6*	*7*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Group I													
May 25	378	6	-	-	-	-	10	-	-	-	-	-	-
June 16	78	-	-	-	-	-	9	-	-	-	-	-	-
July 11	18	9	-	-	-	-	9	9	2	2	-	-	-
Aug 1	4	81		-	-	2	6	27	-	-	-	-	-
Aug 16	6	-	-	-	-	-	8	90	-	-	-	-	-
Sep 5	5	23	-	-	-	-	8	70	-	-	3	-	-
Oct 4	5	50	-	-	-	-	-	30	10	10	-	-	-
Oct 25	-	-	-	-	-	-	-	48	-	-	-	-	-
Group II													
June 16	10	-	-	-	-	-	24	26	-	-	-	-	-
July 11	-	-	-	-	-	-	2	62	-	2	-	-	-
Aug 1	-	-	-	-	-	-	-	76	-	-	-	-	-
Aug 16	5	-	-	-	-	-	-	182	4	2	-	-	-
Sep 5	-	-	-	-	-	-	-	414	13	9	-	-	-
Oct 4	-	-	-	-	-	-	-	-	3	-	-	-	3
Group III													
July 11	-	354	-	-	-	-	-	410	-	-	-	-	-
Aug 1	-	25	-	-	-	-	-	26	-	-	-	-	-
Aug 16	-	388	-	-	-	-	-	285	-	-	-	-	-
Sep 5	-	230	-	-	-	-	-	300	-	-	-	-	-
Oct 4	-	300	-	-	-	-	-	320	-	-	-	-	-
Oct 25	-	-	-	-	-	-	-	310	-	-	-	-	-
Group IV													
Aug 1	-	-	-	-	-	-	-	40	-	-	-	-	-
Aug 16	-	-	-	-	-	-	-	-	-	-	-	2	-
Sep 5	-	48	-	-	-	-	-	160	-	-	-	-	-
Oct 4	-	20	-	-	-	-	-	9	-	-	-	-	-
Oct 25	-	42	15	-	-	-	-	5	18	-	-	-	-
Group V													
Aug 16	-	7	-	-	3	-	3	75	-	3	-	-	-
Sep 5	-	6	-	-	-	-	-	96	-	-	-	-	-
Oct 4	-	9	-	-	-	-	3	76	-	3	-	-	-
Oct 25	-	3	-	3	-	-	-	33	-	-	-	-	-

Table 3. Relative abundance of mollusk species on oyster shell and mussel shell collectors (0 = no occurrence, 5 = full coverage).

	Oyster shell				*Mussel shell*			
Sampling date	*Barnacles*	*Polychaete*	*Bryozoa*	*Ascidiacea*	*Barnacles*	*Polychaete*	*Bryozoa*	*Ascidiacea*
Group I								
May 25	1	-	-	-	5	1	-	-
June 16	2	-	-	-	2	-	-	-
July 11	5	2	-	-	2	1	-	-
Aug 1	4	1	-	-	1	1	1	-
Aug 16	4	1	1	-	1	1	-	1
Sep 5	4	2	1	-	1	1	-	-
Oct 4	5	2	1	-	1	-	-	-
Oct 25	3	1	-	-	1	3	-	-
Group II								
June 16	2	-	-	-	1	1	-	-
July 11	3	-	-	-	2	-	-	-
Aug 1	4	1	-	-	1	2	-	-
Aug 16	3	1	-	-	2	1	-	-
Sep 5	5	-	-	-	1	1	-	-
Oct 4	5	-	-	-	-	-	-	-
Oct 25	-	-	2	1	1	-	-	-
Group III								
July 11	1	-	-	-	1	-	-	-
Aug 1	5	1	-	-	2	1	-	-
Aug 16	5	1	1	-	2	1	-	-
Sep 5	5	3	-	-	1	2	-	-
Oct 4	5	2	1	-	5	-	-	-
Oct 25	5	-	2	-	5	1	-	-
Group IV								
Aug 1	1	1	-	-	2	-	-	-
Aug 16	5	2	-	-	2	-	-	-
Sep 5	4	2	-	-	2	2	-	-
Oct 4	5	1	-	-	2	-	-	-
Oct 25	5	-	1	-	2	1	-	-
Group V								
Aug 16	3	2	-	-	5	2	-	-
Sep 5	5	3	-	-	5	3	-	-
Oct 4	5	1	-	-	4	2	-	-
Oct 25	5	-	-	-	2	1	-	1

a commercial spat set. MacKenzie (1981) used a criterion of 2.5 spat per shell to define a commercially successful oyster set on shells in Long Island Sound. Therefore, it appears that the spat set on the collectors in Karantina Island in our study was within the standards required for commercial use.

Settlement was significantly higher on the outside than on the inside surfaces of the oyster shells. In contrast, settlement was higher on the inside surfaces of mussel shells. The outside surface of oyster shells collects more spat than the inner surface because silt accumulation is higher on the outside (Quayle, 1969). The higher rate of recruitment on the insides of mussel shells may have been due to the lower accumulation of suspended particles or because the concave shape forms an eddy that accumulates oyster larvae (León, 1981). Saoud et al. (2000) reported that the majority of spat in their study settled on the two interior surfaces of the collector plates, with 66% setting on top of the lower plate.

Also in the bivalves *Ostrea lurida* (Hopkins, 1935), *Ostrea gigas* (Schaefer, 1937), *Ostrea edulis* (Cole and Knight-Jones, 1949), *C. rhizophorea* (León, 1981; Buitrago and Alvarado, 2005), and *C. virginica* (Kenny et al., 1990) spat settlement was higher on the under surfaces of the collectors. Dimani and Lenz (1974) concluded that *Saccostrea commercialis* larvae settle on upper surfaces of collectors only when the spat density on the under surfaces is high. Consistently higher spat abundance on the bottom sides of collection plates at the highest level suggests that direct exposure to light and heat adversely affect settlement in the intertidal zone. It appears there is a tendency for a greater abundance of spat on the shaded side of collectors (Hopkins, 1935; Cole and Knight-Jones, 1939; Kenny et al., 1990). Site specific differences in turbidity or siltation can be another factor influencing the preferred side for settlement. The accumulation of silt may render potential settlement surfaces unsuitable or smother newly settled forms (Abbe, 1986). Holiday (1996) reported that settlement of *S. commercialis* was similar on the convex (outside) and concave (inside) surfaces of vertically deployed slats, but settlement was higher on the under surfaces than on the upper surfaces when collectors were deployed horizontally.

Throughout the experiment, 51% and 70% of the settled spat (oyster and mussel shell collectors, respectively) were under 10 mm. Buitrago and Alvarado (2005), who preferred to use 23 mm *C. rhizophorae* for growout following spat collection, reported that 51% of the settled spat was under 10 mm. In our study, 10% and 5% of the spat (oyster and mussel collectors, respectively) were larger than 20 mm.

Among the factors that influence spat attachment, temperature plays a major role by acting chiefly on the triggering and progress of gametogenesis and spawning, and by regulating the abundance of planktonic food necessary for breeding and larvae development (Bonnet and Troadec, 1985). Yolkolu and Lok (2000) reported that the flat oyster gonad was active as early as mid-March when water temperature was 14°C and that the main spawning period of flat oyster in Karantina Island was April-June. In this study, however, no spat attachment before May was observed.

Distribution and abundance of oyster spat on mussel collectors in our study was probably affected by high density of common jingle shells. Fouling is potentially an important factor on spat recruitment success.

The temperature in Karantina Island during the first sampling was higher than that reported by Kenny et al. (1990) but did not affect settlement. It seems that the salinity fluctuation was not a significant factor for settlement and that, in general, settlement was not related to water changes. Kenny et al. (1990) found that the lowest spatfall coincided with unusually high temperatures and salinity in summer 1986 in North Inlet Estuary, South Carolina. Chlorophyll *a* concentration has been identified as a controlling factor in bivalve growth (Page and Ricard, 1990). In this study, chlorophyll *a* varied 1.5-9.55 µg/l and was good for oyster shell growth. Toro et al. (1995) found that chlorophyll *a* of 2.71-4.92 µg/l was good for growth of the Chilean oyster, *Ostrea chilensis*, in Chile.

This study demonstrates that spat collection at Karantina Island, Izmir Bay, has the potential to supply significant numbers of juvenile oysters for future culture operations. Future studies on settlement dynamics may lengthen the settlement period. Growth and survival rates of settled oyster spat should be compared throughout the year.

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