Seasonal Variations in Condition Index and Gonadal Development of the Introduced Blood Cockle *Anadara inaequivalvis* (Bruguiere, 1789) in the Southeastern Black Sea Coast

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Abstract

The reproduction and gonad development of the *Anadara inaequivalvis* was investigated over the course of one year on the samples collected from the coastal waters of the Southeastern Black Sea by dredging and diving to depths between 15 and 20 m. Histological analyses have shown that the rate of female and male is 1:1. Minimum size for spawning was found as 20 mm in May. Spawning season covers the period from June to September. During this period, the gonadal stages consisting of five phases including primordial, immature, mature, spawn, full spent, resting were observed. The results obtained in this study will certainly help to develop the management strategy of this invader in the Black Sea coasts.

Key words: Black Sea, *Anadara inaequivalvis*, reproduction, gonad development sages

Introduction

Marine bivalve molluscs, which are used as source of food and raw material for ornamental industry, are inhabitants of freshwater, brackish, and freshwater around the world. The members of the family Arcidae are the most abundantly occurring species in tropical regions and have very high economic value for the Indo-Pacific Region (Broom, 1985; Kim and Kang, 1987; Narasimham, 1988a; FAO, 1996).

Recently, a member of Arcidae, *Anadara inaequivalvis* (Syn. *Scapharca inaequivalvis*), has been introduced into Black Sea accidentally (Zolotarev and Zolotarev, 1987; Şahin, 1995; Şahin *et al*., 1999). Although marine bivalve mollusks have no substantial demand in domestic market, there is high demand from European and the Far East markets (Şahin, 1995; Alpbaz and Önen, 1991; Tarkan, 1991). For instance production of *Ostrea edulis, Tapes decussatus*, *Venus verrucosa, Venus gallina, Mytilus galloprovicialis* and gastropd *Rapana thomasiana* increased up to 1994 as a result of export demands, but started to decline after that. It is likely that the stocks of Arcidae on Black Sea coasts are going to be exploited in the near future. Therefore, management actions need to be developed for sustainable fisheries. The currently applied management strategies for bivalve species are local and are limited to size and seasonal restrictions, which seem not to be affective tools to protect stocks against over-exploitation. Thus, new management measures for entire Black Sea coasts are needed. Several management strategies can be developed using the biology of species in questions. However, among these biological features, reproductive biology of species is the most widely used for developing management strategies. Here, we aim to determine some management strategies of clam by examining reproductive biology.

Materials and Methods

The samples were collected during March 1998 - February 1999 by dredging and diving in the coastal waters of Trabzon (40º 20'N - 40º 93'E and 40º 28'N - 40º 95'E) at depths of 15-20 m (Figure 1). During sampling events temperature, salinity and dissolved oxygen values were measured at depths of 10-15 m using a YSI-85 multipurpose, while water samples were taken for chlorophyll-a analysis which was determined by spectrophotometer (Shimadzu 120-01) following Strickland and Parson's method (Parson *et al*., 1984; Gicenberg, 1978). Live clam samples were transferred to the laboratory in a cooling box and they were divided into two subgroups for condition index and gonadal development studies. The condition index was estimated using two formulae.

KI_1 = (Dry Meat Weight (g) / Dry Shell Weight (g)) x 100

KI_2 = (Ash Free Dry Meat Weight (g) / Dry Shell Weigh (g)) x 100 (Aldrich and Crowley, 1986; Devenport and Chen, 1987).

Triplicates two grams of tissue samples were dried in an oven at 80-105°C for 24 hours for determination of dry weight. Ash fry dry meat weight was determined in triplicates porcelain cups burning two grams of the tissue samples at 550°C for 3-4 hours in a muffle furnace. Prior to weighing, all samples were cooled in a desiccator (Aldrich and Crowley, 1986; Devenport and Chen, 1987; Okumuş, 1993). Seasonal (monthly) variations in lipid levels were determined after Soxhelet extraction of the dried

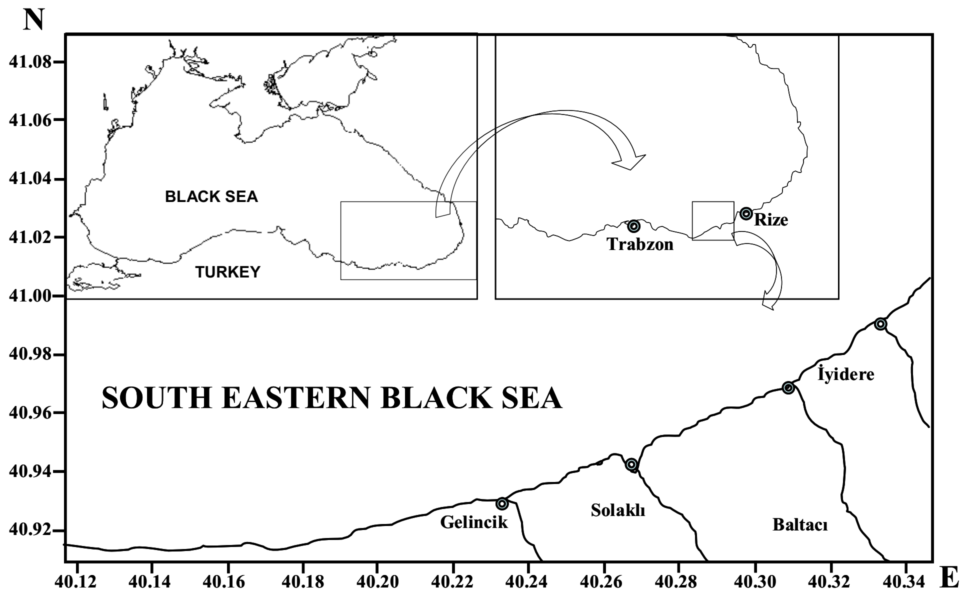


Figure 1. The study area.

tissues with petroleum ether for 24 h, while protein content was derived using micro Kjeldahl method (AOAC, 1990; Iglesias and Navarro, 1991; Bligh and Dyar, 1984; Sedano *et al.*, 1995; Dare and Edwards, 1975). The relationships between both biological (chlorophyll a, condition index, dry meat etc.) and environmental variables (temperature, salinity etc.) were analyzed using a partial correlation procedure.

Monthly samples were grouped to 15 length groups with 5 mm intervals and 5 individuals were sampled from each group for the histological studies. Skins were removed and samples fixed in Bouin's solution and then immersed in paraffin bath to take cross-sections about 7-10µ thickness in various directions. Sections then were stained with hematoxylene and eosin. Gonad development stages were estimated observing sections under the microscope as resting, beginning, developing, maturing, spent, and full spent phases (Smith, 1968; Lange, 1987; Dzyuba and Maslennikova, 1982; Navarao *et al.*, 1989, Morton, 1990).

Results

The mean monthly variations in temperature, dissolved oxygen, chlorophyll and salinity are given in Figure 2. Minimum and maximum temperatures were recorded during July (23.5ºC) and February (11.2ºC). Salinity varied from 17 to 19‰ during the sampling period, while dissolved oxygen was the highest in April and May (7.6 mg/l) and the lowest in June (5.3 mg/l) when temperature was high. Chlorophyll-a content reached maximum of 1.085 mg/l during April and minimum (0.183 mg/l) in July (Figure 2). Partial correlation analysis indicated that both of the condition indexes (KI_1 , KI_2) were significantly correlated with chlorophyll-a values (r=0.67 and r=0.66 for KI_1 and KI_2 respectively).

Both condition indexes exhibited clear seasonal cycle (Figure 3). The monthly condition index values obtained using both methods were the highest of the year in May (KI_1 = 11.266±0.667 and KI_2 = 10.45±0.588) and the lowest in August (4.675±0.240 and 4.265±0.219, respectively).

Biochemical composition of tissue, particularly water, protein and lipid, showed a clear seasonal variation as well (Figure 4). Water content of the tissue varied between 75.3% and 83.7% reaching maximum level in the mid-summer and minimum levels during spring months. The mean protein percentage was the highest in May (60.27%) and the lowest in July (53.91%). The lipid level, on the other hand, was the highest in March (10.38%) and dropped a minimum value in June (6.75 %). Ash content was quite steady fluctuating between 1.4% and 2.2% during the year (Figure 4).

Sex Ratio and Gonadal Development

The first sex discrimination was performed during May. The estimated sex ratio was 1:1 (Figure 5). The minimum size of sexually mature females was about 20 mm.

Gonads of all the specimens captured during March and April were at the (primordial) resting phase (I), which made determination of the sex impossible (Table 1, Table 2) (Figure 6-f, Figure 7-f). However, sex discrimination was possible and conducted in and after May.

Of the 50 specimens collected during May, 23 of them were female and 25 males. Gonads of these females were mostly (61%) in the second or

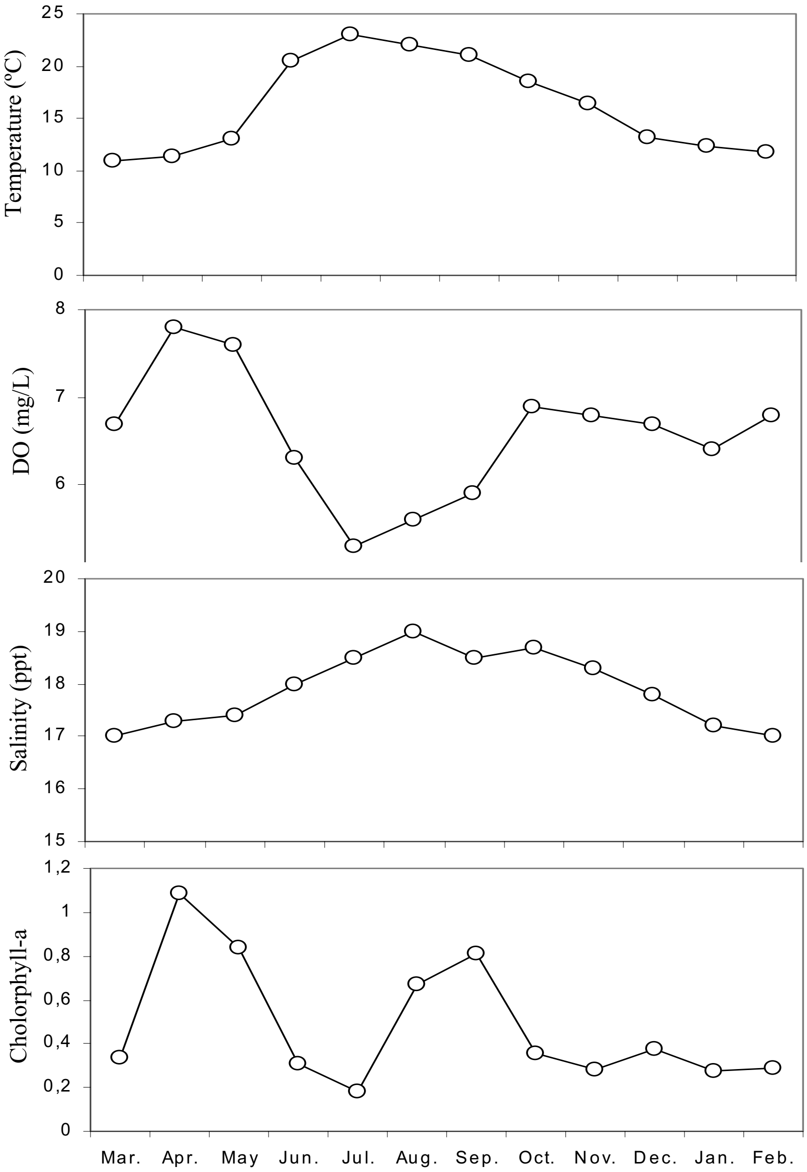


Figure 2. Monthly variations in temperature, dissolved oxygen, salinity, and chlorophyll of the study area.

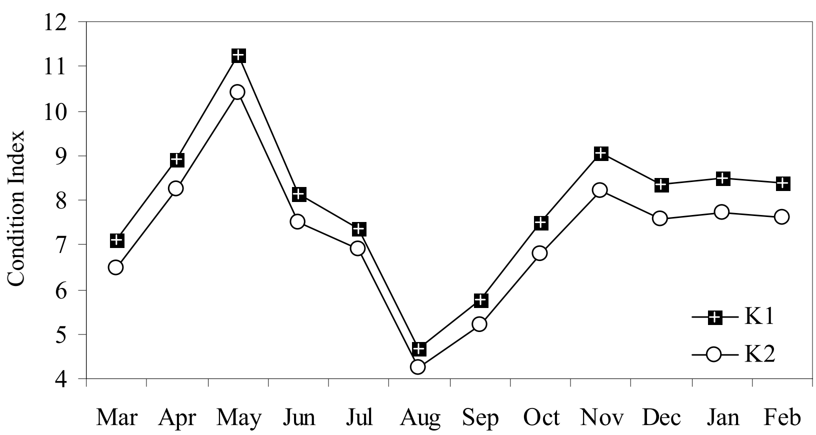


Figure 3. Monthly variations in two condition indexes.

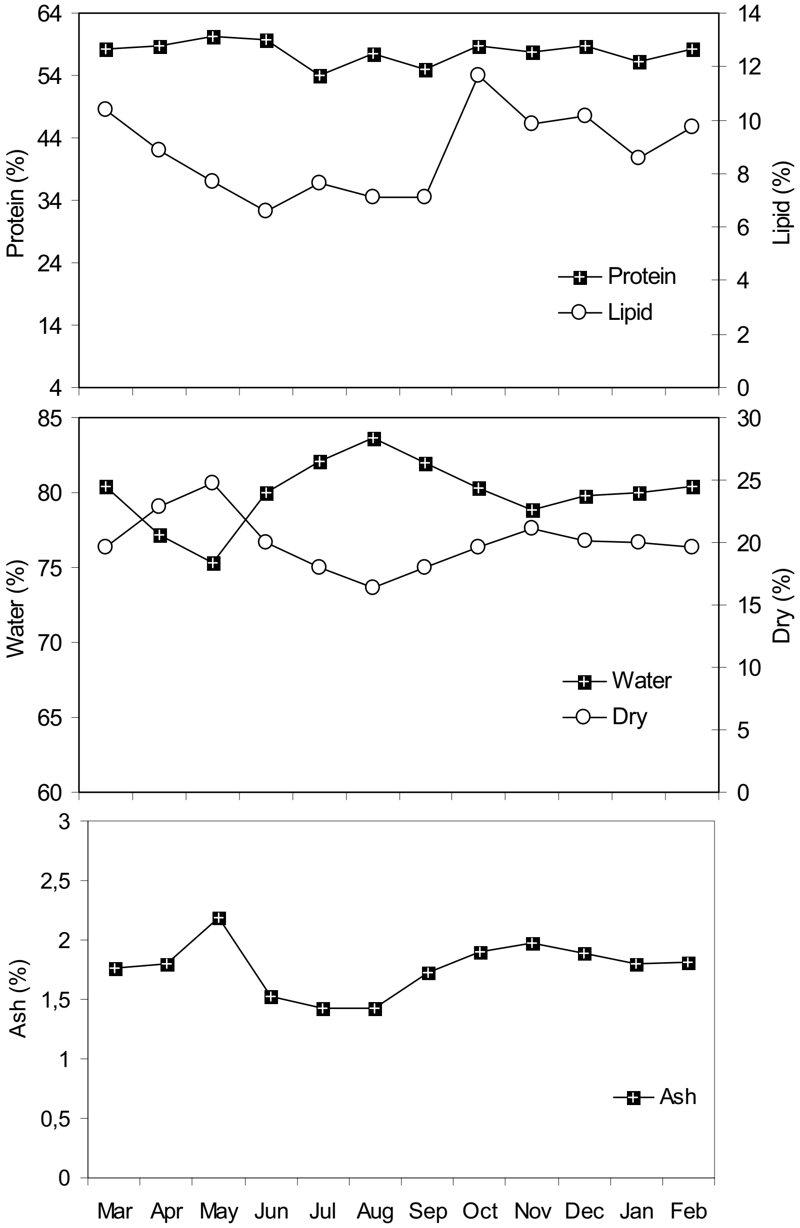


Figure 4. Monthly variations at the proximate levels from March 1998 to February 1999.

beginning phase, while considerable part (34%) in developing (III) and only 5% was in the maturing (IV) phase. Male testis exhibited similar pattern during this month; 64% the beginning (II), 34% developing (III) and 12% maturing (IV) phases (Figure 6-a, 6-b; Figure 7-a, 7-b). Beginning phase of gonad development was completed in great extent during June leaving only 19% of the females at phase II. Rates of females at development phases of III, IV and V were 38%, 30% and 19%, respectively in June. Males exhibited very similar pattern of gonadal development (Table 2; Figure 6c, 6d; Figure 7c, 6d).

First spawning (egg release) was observed during June. Most of the females (55%) and males (67%) during July were at spent phase (V). During August, more than half of the females were at spent (V) and one third and full spent phase (VI) (Table 1), while almost half of the males was at spent (V) and 40% of them full spent phases (Table 2; Figure 6-e, Figure 7-e). During September nearly half of females (44%) and males (48%) were at resting phase, these ratios increased to 90% in October. All females and males were at the resting phase from November to February (Table 1, 2).

Discussion

Aquatic animals are under the effects of both abiotic (e.g. temperature, salinity, dissolved oxygen and substratum) and biotic (e.g. food, predation and

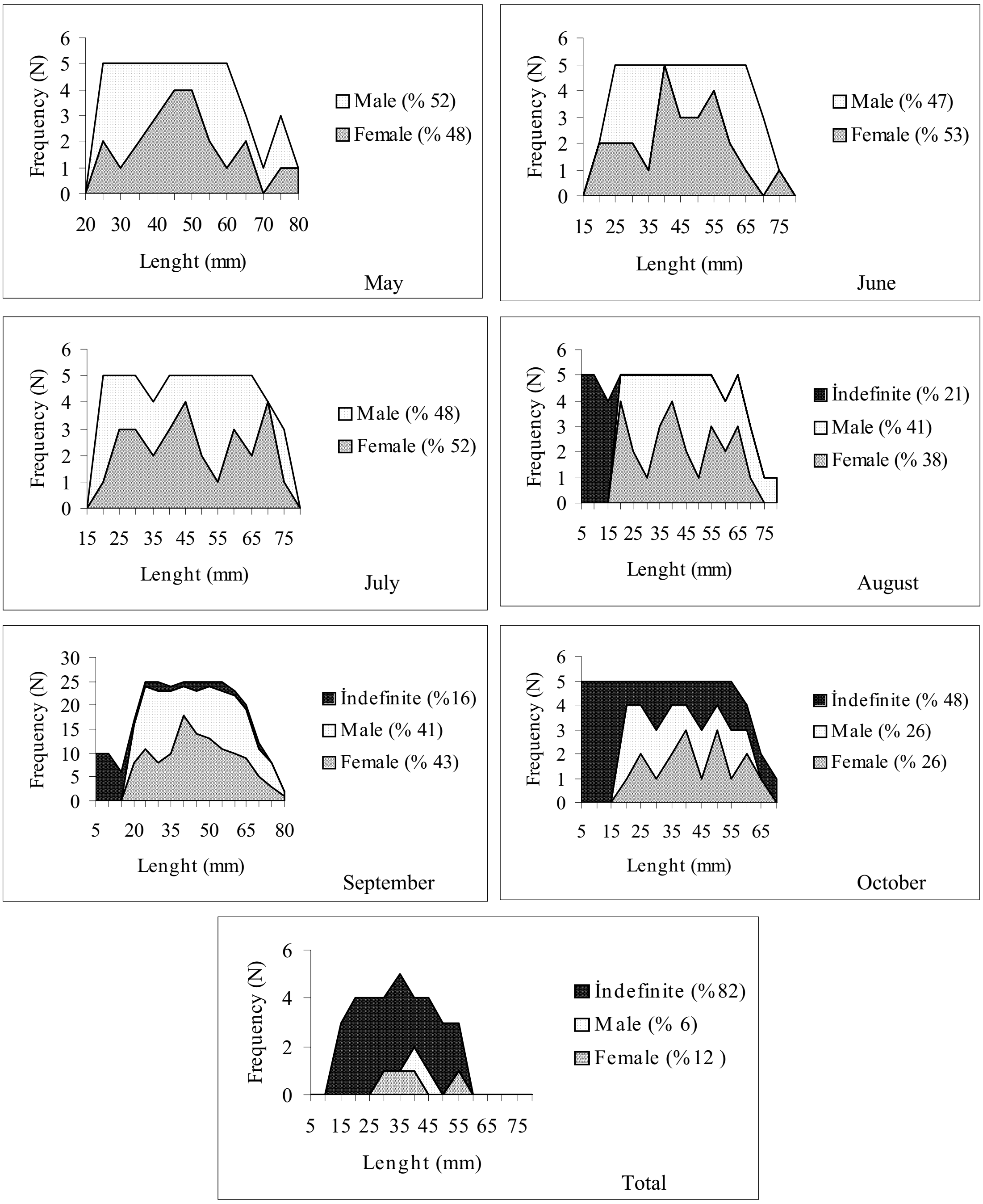


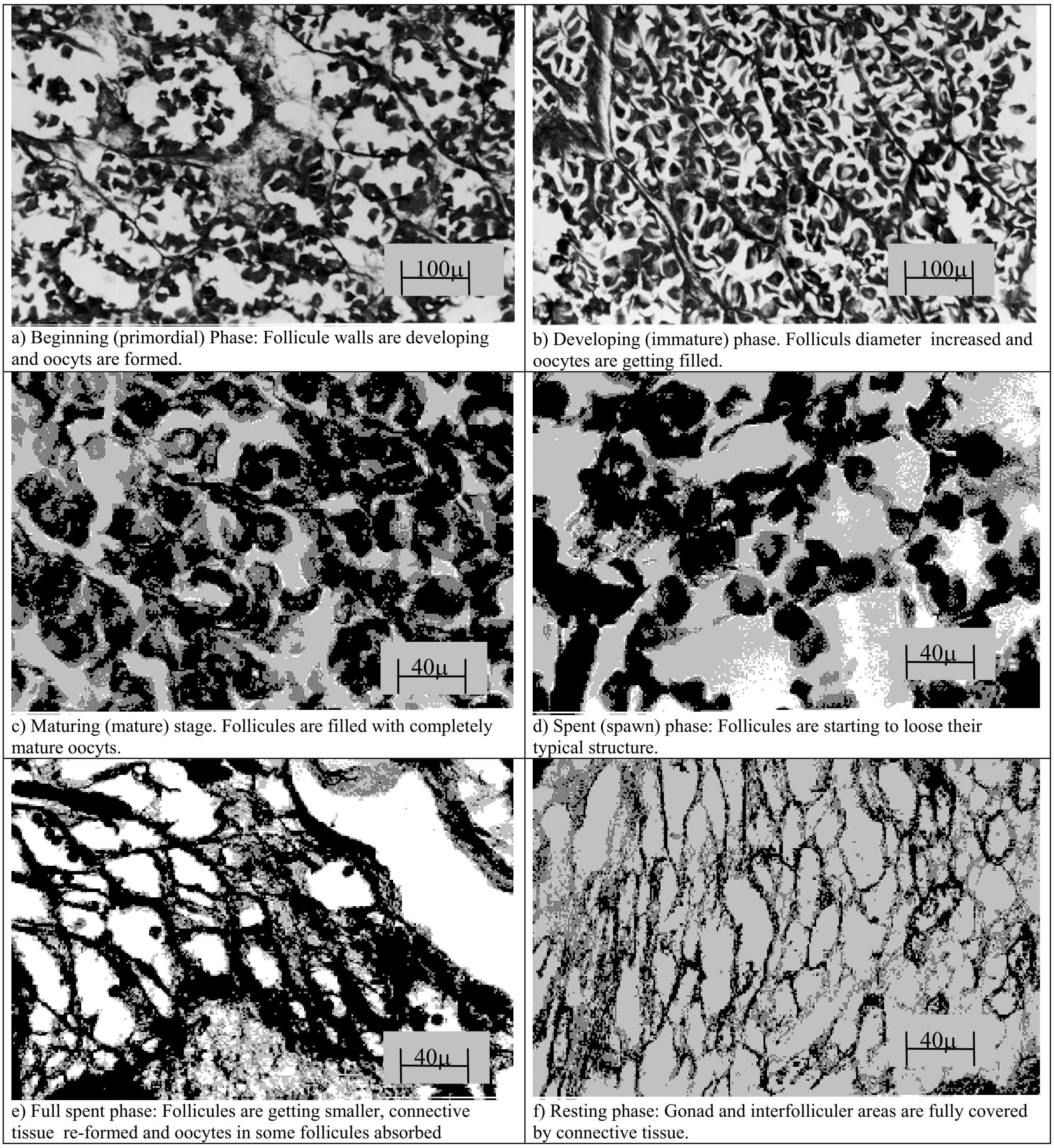
Figure 5. Distribution of sexes by months.

Table 1 . Gonadal development phases of females and percentage distribution

Months	Female	Uncertain	I		II		III		IV		V		VI	
			N	%	N	%	N	%	N	%	N	%	N	%
March	-	20	20	100	-	-	-	-	-	-	-	-	-	-
April	-	20	20	100	-	-	-	-	-	-	-	-	-	-
May	23	-	-	-	14	61	8	34	1	5	-	-	-	-
June	27	-	-	-	5	19	10	38	7	30	5	19	-	-
July	29	-	-	-	-	-	2	7	11	38	16	55	-	-
August	26	-	-	-	-	-	-	-	2	8	16	61	8	31
September	18	14	14	44	-	-	-	-	-	-	7	22	11	34
October	4	28	28	88	-	-	-	-	-	-	-		4	12
November	-	20	20	100	-	-	-	-	-	-	-	-	-	-
December	-	20	20	100	-	-	-	-	-	-	-	-	-	-
January	-	20	20	100	-	-	-	-	-	-	-	-	-	-
February	-	20	20	100	-	-	-	-	-	-	-	-	-	-

Table 2. Gonadal development phases of males and percentage distribution

Months	Male	Uncertain	I		II		III		IV		V		VI	
			N	%	N	%	N	%	N	%	N	%	N	%
March	-	20	20	100	-	-	-	-	-	-	-	-	-	-
April	-	20	20	100	-	-	-	-	-	-	-	-	-	-
May	25	-	-	-	16	64	6	24	3	12	-	-	-	-
June	24	-	-	-	3	13	7	28	10	42	4	17	-	-
July	27	-	-	-	-	-	3	11	8	30	18	67	-	-
August	28	-	-	-	-	-	-	-	4	14	13	46	11	40
September	15	14	14	48	-	-	-	-	-	-	6	21	9	31
October	2	28	28	93	-	-	-	-	-	-	-		2	7
November	-	20	20	100	-	-	-	-	-	-	-	-	-	-
December	-	20	20	100	-	-	-	-	-	-	-	-	-	-
January	-	20	20	100	-	-	-	-	-	-	-	-	-	-
February	-	20	20	100	-	-	-	-	-	-	-	-	-	-



a) Beginning (primordial) Phase: Follicule walls are developing and oocyts are formed.

b) Developing (immature) phase. Folliculs diameter increased and oocytes are getting filled.

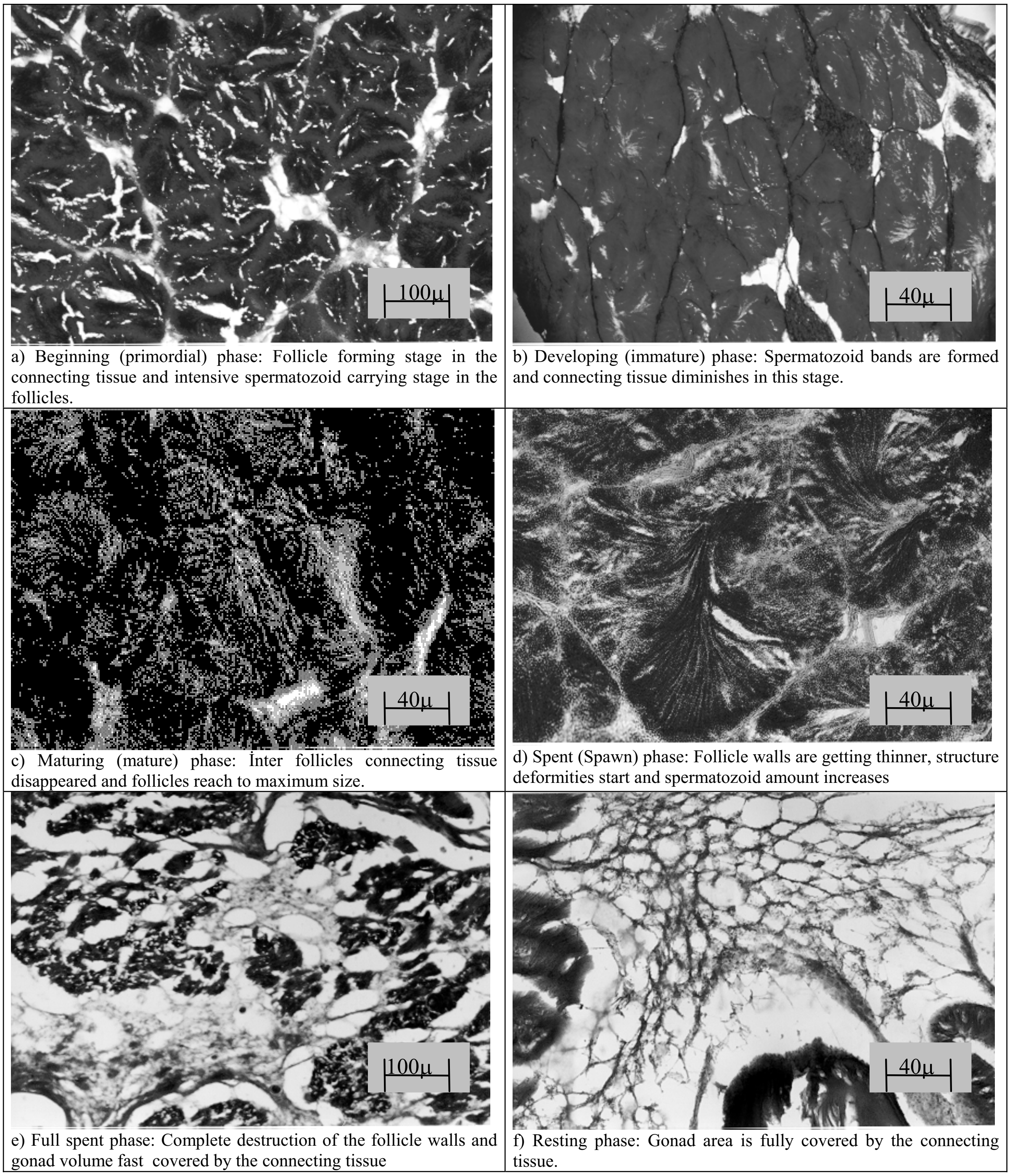
c) Maturing (mature) stage. Follicules are filled with completely mature oocyts.

d) Spent (spawn) phase: Follicules are starting to loose their typical structure.

e) Full spent phase: Follicules are getting smaller, connective tissue re-formed and oocytes in some follicules absorbed

f) Resting phase: Gonad and interfolliculer areas are fully covered by connective tissue.

Figure 6. Gonad development phases in females.



a) Beginning (primordial) phase: Follicle forming stage in the connecting tissue and intensive spermatozoid carrying stage in the follicles.

b) Developing (immature) phase: Spermatozoid bands are formed and connecting tissue diminishes in this stage.

c) Maturing (mature) phase: İnter follicles connecting tissue disappeared and follicles reach to maximum size.

d) Spent (Spawn) phase: Follicle walls are getting thinner, structure deformities start and spermatozoid amount increases

e) Full spent phase: Complete destruction of the follicle walls and gonad volume fast covered by the connecting tissue

f) Resting phase: Gonad area is fully covered by the connecting tissue.

Figure 7. Gonad development phases in males.

competition) variables. All the environmental variables may affect the growth and reproduction as well as the distribution of *Anadara inaequivalvis*. Among the biotic variables the chlorophyll-a, an indicator of phytoplankton biomass and a very well known source of food for bivalve molluscs, was highly correlated with condition index during the present study. Highest levels of chlorophyll-a content and condition index matched very well during April – May. It is known that good condition values are the indicators of accumulating nutrient reserves, particularly glycogen and protein, and gonadal development (Dare and Edwards, 1975; Bligh and Dyar, 1984; Aldrich and Crowley, 1986) . In present study, two peaks in condition index were observed during annual cycle (Figure 3); major one in May and second one in November. Gradually decreasing condition index from May to August indicated significant loses in tissue weight or reserves due to release of gametes. After August condition index started to rise gradually reaching second peak in November when autumn blooms occurs in the area. This is a typical seasonal cycle observed in bivalves in subtropical and temperate regions (Iglesias and

Navarro, 1991).

It is reported that there have been important changes in the metabolic activities in pre/post reproduction period of the bivalve molluscs (Okumuş, 1993; Moscose *et al*., 1992; Narasimham, 1988b). Significant declines in protein and lipid levels are inevitable during spawning season. In contrast reserves such as glycogen and lipid are utilized during the winter when available food is limited. Seasonal variation in condition index also reflects this fact (Figure 4) (Aldrich and Crowley, 1986; Devenport and Chen, 1987; Okumuş, 1993). The results of the present study also exhibited similar pattern. The lipid content reached maximum levels during autumn and was used as energy reserve during winter. During the spring gonadal development caused slight increment in protein content but not in lipid. That is because lipid was used as energy source for accelerating metabolism due to increasing temperature and gametogenesis (Figure 4).

Observation of histological samples of gonads indicated that spawning in the Black Sea population of *A. inaequivalvis* took place during summer period. Native bivalve species in the Black Sea spawn earlier than *A. inaequivalvis*. It is most likely that *A. inaequivalvis* as a tropical species needs higher temperatures for spawning and larval development. Gonadal development stages for other species of Anadara (i.e., *A. granosa* and *A. rehombea*) from various localities have been described as resting, beginning, developing, maturing and spent and differentiations between these phases seem to quite similar (Dzyuba, 1982). Studies on *A. subcrenata*, *A. broughtoni* and *A. ursi* in sub-tropics showed that spawning took place from June to September (Morton, 1990; Moscose *et al*., 1992; Dzyuba and Maslennikova, 1982; Cruz, 1984; Yankson, 1982; Hadfield, and Anderson, 1988; Cruz, 1987; Trol and Gomez, 1985). During the present study females with mature gametes were found until September and October, but their ratios declined rapidly after August (Table 1, Table 2).

Sex ratios of the specimens sampled were determined monthly from the histological slides (Figure 5) as 1.04:1 (F/M) and χ^2 has shown that there is no significant difference between sex ratio. The sex ratio for other Anadara populations is given in Table 3. It can be clearly seen that there is good agreement with finding of present study (Table 3).

Determination of first maturity length and spawning period are the basic requirements for the protection and sustainable exploitation of the stocks. There are several methods used for studying these variables, however the most precise results have obtained through histological examinations. It is almost impossible to determine the beginning (II) period of the gonadal development using other approaches. In conclusion, the introduced bivalve species has been adapted into new environment exhibiting similar seasonal cycles in condition index and gametogenesis with other species of the genus Anadara inhabiting sub-tropic and temperate waters and other bivalve species in the Black Sea.

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Table 3. Sex ratios in some Anadar*a* species

Country	Species	Sex Ratio (F/M)	Researchers
India	*A. granosa*	1:1	Narasimham, 1988
Costa Rica	*A. tuberculosa*	1:1	Cruz, 1984
Philippines	*A. antiquata*	1.03:1	Toral, 1985
East Africa	*A. senilis*	1.2:1	Yankson, 1982
Australia	*A. trabezia*	1:1	Hadfield, 1988
Costa Rica	*A. grandis*	1.2:1	Cruz, 1987
Turkey	*A. inaequivalvis*	1.04:1	This research

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