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The ecology of two species of blood clams, Anadara granosa (L.) and Anadara antiquata (L.) in Central Java, Indonesia.

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THE ECOLOGY OF TWO SPECIES OF BLOOD CLAMS *Anadara granosa* (L.) AND *Anadara antiquata* (L.) IN CENTRAL JAVA, INDONESIA

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To my mother S.A. Brotohadikusumo and
my father the late R.M. Brotohadikusumo

SUMMARY

Members of the family Arcidae (phylum Mollusca, Class Bivalvia) typically occur in habitats ranging from the intertidal zone on wave-exposed sandy shores to the marginally subtidal areas of sheltered mudflats bordering mangroves. They also occur at much higher tidal levels within the mangrove system (e.g. tambaks) and even extend into deeper subtidal areas. This study confirms that *Anadara granosa* possesses remarkable behavioural and physiological adaptations to deal with short-term fluctuations in environmental conditions.

Both *A. granosa* and *A. antiquata* are gonochoristic species. Planktotrophic post-fertilisation development is associated with the production of large numbers of small (45-65μm) eggs during their iteroparous life cycle. A few individuals were reported with both male and female gametes present within the same individual follicles; these appear to be sequential protandric hermaphrodites with only a single sex change during their life history. However, *A. antiquata* grows to a larger size prior to the onset of reproduction. *A. granosa* exhibited major spawnings during July-September 1992 and June-August 1993 with other spawnings in February and March 1992 and 1993.

Allometric growth in both *A. granosa* and *A. antiquata* seems to be associated with the development of a broad, light weight shell necessary for life in relatively soft sediments. Two distinct ecomorphs of *A. granosa*, one rounded and one elongated form, are described. Although the growth bands within the shell of *A. antiquata* are difficult to interpret, those in *A. granosa* are clearly defined and have been shown to have a tidal periodicity which reflects the predominantly diurnal component of the mixed semidiurnal tidal regime. These growth bands exhibit a semilunar pattern in which narrower growth increments are produced during or a few days after spring tides whilst wider increments are produced during neap tides. The width of the growth increments was also shown to vary seasonally with groups of narrow increments deposited during February-March and July-September when the clams were spawning, whilst the widest increments were laid down during May-June. This pattern of narrow and wide increments was used to estimate the age of individual clams and the age composition of the population. Whereas the population of *A. granosa* at Wedung consited of younger clams (1-4 years old) the population at Tapak consisted mainly of older (6-12 years old) clams.

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CHAPTER I

GENERAL INTRODUCTION

Marine bivalve molluscs of the family Arcidae, subfamily Anadarinae, mostly have solid shells and are generally trapezoidal in outline varying from sub-ovate to quadrate. They may be characterised by being inequivalve. However, this character is variable, appearing in juveniles only in some species and being present or absent in adults of others (Oliver, 1992). The shell is unequilateral in shape with the beaks (umbones) normally in the anterior half of the shell. This group still retains some primitive characters which are present in all species; these include the distinctive duplivincular ligament which is shared only with the Glycymerididae, and a row of many small alternating taxodont teeth and sockets in the hinge plate of each valve, which increase in size toward the margins. The duplivincular ligament takes the form of a series of lamellar bands stretched across the cardinal area between the umbones which may be widely separated. Typically the ligament is placed obliquely giving a chevroned appearance.

It was thought that, being amongst the least modified byssally attached bivalves, ark shells had probably been compelled to retain the primitive isomyarian condition (two adductor muscles of similar size) by virtue of their exceedingly weak ligament (Thomas, 1976). Furthermore, unlike most bivalves, the mantle isthmus of these arcids is divided into two during ontogeny with the outer marginal folds of the left and right mantle lobes running across the middle (Owen, 1959). The scars of the adductor muscles

are slightly subequal in size, the pallial line is entire and the inner margin may be smooth or deeply crenulated (Tebble, 1966; Oliver 1992).

Some species of Anadarinae, particularly those epifaunal ones which attach to rocks, have a byssal system. In exposed intertidal habitats some species are almost mytiliform with a large, strong byssus such as *Barbatia barbata* (L.) and these contrast with those from sheltered crevice sites where the outline is rectangular and the byssus greatly reduced. Infaunal species either live freely unattached, e.g. *Anadara granosa* (L.) or posses a weak byssus of narrow bands like those seen in *Anadara antiquata* (L.). As a group they are poor burrowers, some living only partly buried.

The genus *Anadara* is geologically fairly recent, albeit not the youngest when compared with many other genera within the family Arcidae which have representative forms like *Barbatia* that originated in the Triassic period. The earliest species of *Anadara* existed in the Tongrian Stage of the Oligocene period, some 26 million years ago. This species was first described as *Arca sulcicosta* (Nyst, 1836 cited in Lim 1968) but was subsequently placed in the genus *Anadara* by Gray (1847 cited in Lim, 1968). *Anadara* began with a few Oligocene species, such as *A. invidiosa* (Cassy), *A. sulcicosta* (Nyst), *A. waylandi* Cox, and *A. daitokudoensis* (Makiyama); which were widely distributed in North America, Europe, Africa and Japan respectively. Moreover, the number of species within the genus *Anadara* increased during the Miocene and later geological periods (Lim, 1968).

The recent genus which has five living subgenera a) *Anadara s.s* b) *Scapharca* c) *Cunearca* d) *Argina* e) *Senilia* (Reinhart, 1935 in Yoloye, 1975; Lim, 1968) and contains about sixty species, is distributed as far

south as southern Australia and as far north as the islands of Japan and the Mediterranean Sea. Anadarinids are typically found in estuarine and brackish water environments and occur from the intertidal zone to subtidal depths of 80-100 fathoms (Lim, 1968).

Members of the subfamily Anadarinae are frequently termed "cockles" as morphologically they superficially resemble the European cockle *Cerastoderma edule* which in fact belongs to the family Cardiidae. This terminology, therefore, does not have any taxonomic significance. *A. granosa* is sometimes called the mangrove cockle (Borrero, 1980) because of its association with mangroves, or the blood/bloody cockle (Wong et al, 1985; Davenport and Wong, 1986) because of the high levels of haemoglobin in the blood. Anadarinids have also been called arc/ark-shells (Tebble, 1966) or blood clams (Patel and Eapen, 1989a, b). Further in the text, these two later terminologies will be applied for the two species studied, i.e. *A. granosa* and *A. antiquata.* Unlike most other bivalve species, the genus *Anadara* is specialised with the invariable occurrence of the red blood pigment haemoglobin in all of its species, hence the common name blood clam (Wong et al, 1985; Davenport and Wong, 1986; Patel and Eapen, 1989a, b). So far the largest recorded ark shell is *A. grandis* off the Colombian coast, the largest specimen of which weighed more than 1 kg (Squires et al, 1975).

A. granosa is one of several species within the genus *Anadara* found in Indonesian waters and indeed is the only one regularly collected as food among people throughout the archipelago. This blood clam has an equivalve subquadrate outline, the anterior margin of which is broadly rounded whilst the posterior margin is slightly expanded. The umbones are located just in front of the mid line. *A. granosa* has approximately 19 to 21 strong radial

ribs, these broadening over the posterior slope, each with a row of tubercles. The periostracum is dark reddish-brown and usually worn away at the umbones, whilst the cardinal areas are black and the ligament bands arranged obliquely to the hinge line. The inner margin of the shell is deeply crenulated and interlocking whilst the inside of the shell is chalky white.

The other anadarinid species under investigation in this study is *A. antiquata*. According to Lim (1968) and Oliver (1992), *A. antiquata* is the type species for the genus *Anadara* and yet there are few published studies of this species. It has a somewhat elongate subrectangular outer shape, longer than high, its posterior margin longer and gently sloping. The ligament lacks all but the outer chevron whilst the interior of the shell varies from chalky white to dark green in colour. The sculpture consists of about 35 wide ribs in which the interspaces are narrower than the ribs. Troughs in the weakly nodulose radial ribs are hairy, particularly toward the ventral margin of the shell. The periostracum which is persistent particularly at the margins, consists of fine lamellae and fine erect bristles and is blackish-brown in colour. The umbones which are in the anterior third of the hinge line are not elevated and are rarely worn away due to their life habit of attaching to gravel or pieces of dead coral just beneath the sediment surface. The cardinal area between the umbones is black. The ventral margin of the shell is crenulated, slightly interlocking at the posterior margin and bears no noticeable gape for the insertion of the flattened slim byssus stem. In juvenile specimens (15-20mm length), the two valves are slightly unequal, the right one being somewhat larger. However, with increasing size this difference becomes less evident. As in other Anadarinae, the mantle lobes are free ventrally, the short 'siphons'

being formed by apposition of the inner fold of the mantle margin, wherein whitish light-sensitive pigment spots occur (Lim, 1966).

Recorded data on mortality of *A. granosa* in either natural or artificially seeded beds have usually been attributed to such physical and biological factors as: 1) sudden dramatic depressions of salinity due to periods of prolonged heavy rainfall (Liong, 1979); 2) wave and wind action (Narasimham, 1969; Nair, 1986), 3) shore elevation (Broom, 1983c), 4) gastropod, starfish (Broom, 1983c) and crab predation (pers. obs.). None of the published literature mentions mortality of *A. granosa* due to parasitism or bacteriological causes. Bardach et al (1972) stated that at least up to 1972 no known cases of mass mortality caused by diseases or parasitism had been reported and subsequently there have been no further references until this present study.

Shell-crushing predation by decapod crustaceans has been shown to be an important process in the evolution of mollusc shell and crab claw design (Vermeij, 1977; Zipser & Vermeij, 1978). This coevolutionary relationship has been shown to vary latitudinally with heavier predation pressure by crabs in tropical latitudes (Vermeij, 1977), leading to heavier shells and shell armour in gastropods (Bertness & Cunningham, 1981). Similarly, tropical bivalves have more complete shell protection provided by tight valve closure which is thought to reflect an equatorward increase in crushing predation intensity (Vermeij & Veil, 1978). Meanwhile tropical crabs have been reported as being able to take larger prey than temperate crabs of comparable size (Vermeij, 1976). The latter were found to be less specialized for crushing hard-shelled prey (Zipser & Vermeij, 1978).

The critical size of a prey species is determined both by its own geometrical characteristics, e.g. shape and thicknes of shell, hardness and structure of shell material; as well as by the behaviour and morphology of the crabs. Both *A. granosa* and *A. antiquata* present an approximately globular-outlined shell with interlocking valve margins. Although both blood clams have relatively thicker shells compared to, for example mytilids, which have often been used as the prey model in studying feeding behaviour of crabs (Seed, 1982; 1990a,b; Lin, 1990, Seed, 1993) shell thickness may vary from one subpopulation to another. Difference in shell thickness however, are thought to be due to excessive salinity fluctuations (Broom, 1982a). In the present study, no attempt was made to study the behaviour of both species when exposed to crab predation.

Many species of *Anadara* are gathered for human consumption (Oliver, 1989). On the Pacific coast of Colombia, *A. tuberculosa* (Sowerby), *A. similis* (C.B. Adams), *A. multicostata* (Sowerby) and *A. (Grandiarca) grandis* (Broderip & Sowerby) are all harvested on a subsistence basis (Squires et al, 1975) as is *A. cornea* (L.) in West Africa (Okera, 1976) and *A. antiquata* in Central Java, Indonesia. Yet in the Philippines, *A. antiquata* forms quite an important fishery item in many shoreline towns where this is the only edible bivalve mollusc commercially harvested from wild populations (Toral-Barza and Gomez, 1985). Species harvested on an intensive commercial basis include *A. granosa* in Indonesia, Malaysia and Thailand, *A. subcrenata* (Lischke) in Japan and *A. broughtoni* (Schrenck) in South Korea. There is some culture of *A. granosa* in Malaysia and Thailand (Broom, 1982c; Ng, 1984).

In Malaysia, the early development of blood clam (*A. granosa*) culture has been reported to have begun in 1948 (Pathansali and Soong, 1958). At the present time in Malaysia, the Fisheries Department monitors the growth of spat in their natural beds. When the spatfall is within a size range of 4-10mm the Department authorises the collection of blood clams larger than the minimum legal size of 6.4 mm (Akta Perikanan Malaysia, 1963). The fishermen collect the spat manually at low tide, when the water is about 60 cm deep. The season for collection may continue for several weeks after settlement has been detected and the collected spat are sold both locally and to culturists in Thailand. Usually the culture beds are located both outside and in the mouths of large rivers. The latter beds carry with them the risk of sudden large-scale mortality during prolonged periods of heavy rain (Liong, 1979). The culture grounds are not normally prepared in any special way. The spats are sown at densities up to 2,000 m^{-2}, but are then thinned down as they grow. The larger specimens are removed and resown at densities of 200 - 300 m^{-2}. Growth is monitored and after 6 - 9 months harvesting begins. The minimum legal size for sale in Malaysia is 31.8 mm. The whole bed is usually fished out 12-15 months after seeding.

Tookwinas (1985) reported that culture of *A. granosa* in Thailand first took place about 75 years year ago, in Petchaburi Province. Natural spats were collected and reseeded in bamboo-fenced areas covering 8-16km^2. The culture period was one to two years. Culture continued in this fashion until 1972, when it was brought to a halt by severe pollution in the inner of Gulf of Thailand. In 1973 however, culturists from Satun Province began importing spat from Malaysia to restart the culture operations. Since then harvesting of the blood clams takes place about 18 months after seeding. This is later than

in Malaysia, but is due to the fact that marketable size in Thailand occurs at a length of about 40mm compared to the smaller size of 31.8mm in Malaysia.

In Taiwan *A. granosa* is a highly priced species because of its scarcity and its red meat. Even though they can be found on mud flats inside estuarine or intertidally, their production is insufficient to meet the local demand (Chen, 1984). Thus every year great quantities of blood clams are imported from Korea for temporary stocking to get a good price. The culture practice involves growing the spat in nursery ponds of 30-50cm water depth fertilised with chicken droppings. The water of the ponds should be kept clear or light brown in colour, and the salinity within the range of 20-30‰. The clams are harvested manually using an iron rake. The total annual production for *A. granosa* in Taiwan has dramatically decreased. In 1970 it was 145 metric tons but this had fallen down to less than 3 metric tons in 1982 (Chen, 1984). Overfishing of adult blood clams, short supply of spat and industrial pollution were amongst the reasons responsible for this decline.

In Ketapang, West Java, Indonesia, similar methods to those used in Thailand have been applied since the 1950's, but only when there is heavy spatfall. The culture grounds are established openly in bays, not in sheltered areas where three rivers discharge into it. The practice is essentially an extensive culture method involving collection of spat followed by reseeding the culture ground covering an area of 10-20km^2 (Ismail, 1971). Sowing density was not reported quantitatively, but has been described as 'very crowded'. Since 1967, the last good season for *A. granosa* in Ketapang was in 1970. Spat usually appear in May-June within a size range of 2-5 mm. The culture period however, was only 6-7 months until December-January when rough waves during the monsoon periods tended to ruin the bamboo-fenced

culture ground. This is presumably because the beds were laid unprotected in an open bay. After this short culture period, the blood clam when harvested were on average only 18.3 mm in size (20-25 mm size range; Ismail, 1971).

Artificial induction of spawning and the subsequent rearing of spat has only been achieved with any degree of success for *A. broughtoni, A. subcrenata* and *A. granosa* (Broom, 1985). The latter species based on a single study by Wong et al (1985). The scientific basis of artificial culture of a temperate species *A. broughtoni* is summarised in Broom (1985) from scattered information provided by Kanno and Kikuchi (1962), Kanno (1963), Yoo (1969) and Kim and Koo (1973). Basically, the authors demonstrated that repeated thermal stimulation (Kanno and Kikuchi, 1962, Kanno, 1963) could trigger spawning. Kim and Koo (1973) induced spawning of ripe specimens of *A. broughtoni* by raising the water temperature from 18°C to 28 °C every two hours. Similarly Yoo (1969), kept individuals at a controlled temperature of 18-20°C then subjected them to an induction temperature of 25-29°C.

In all studies, the spawners were collected from natural populations during the breeding season. Following fertilisation, the D-shaped larval stage was apparently attained after 20-24 hours at a water temperature of 20-25°C. Subsequent growth to settlement however, depends on water temperature and on the quality as well as quantity of food provided. Kanno (1963) provided a diet of *Monas sp.* at 1,200-5,400 cells.ml^{-1} at a temperature of 23.8-27°C and found that larvae of *A. broughtoni* grew from 90μm in length to settlement size, 235μm long, in one month. Kim and Koo (1973) fed D-shaped larvae on *Chaetoceros calcitrans, Monochrysis lutheri, Monas sp.* and *Skeletonema costatum.* They varied the feeding such that more food was provided as the

costatum. They varied the feeding such that more food was provided as the larvae grew. The feeding rates were 7,000 cells.larva^{-1}.day^{-1} for the D-shaped veliger, 12,000 for the umbo stage and 23,000 thereafter. Growth however, appears to have been slightly less than that reported by Kanno (1963), a length of 225μm only being achieved after 40 days.

Attachment of post larvae is reported as having taken place at sizes between 180 and 270μm. Yoo (1969) maintained larvae at 20.4 ± 1.7°C and found the best growth was obtained by modifying the quantity of food according to the species of alga and the volume of the tank rather than the number of larvae. Thus, for *Monochrysis lutheri* and *Cyclatella nana* the concentrations in the tank should be 10^4-10^6 cells.ml^{-1}. For *Chaetoceros calcitrans* it should be $20x10^3$-$150x10^3$ cells.ml^{-1}. The author also recommended that the cells should be cleaned (presumably by washing with sterile water) after spinning down in a centrifuge prior to inoculation. In this latter experiment larvae began to settle at a length of 260-290μm, 30 days following fertilisation.

Mortality during the larval rearing period is considerable. Kim and Koo (1973) maintained that only 5% of the larvae survived over the 40 days during the period of the experiment. Somewhat better results were obtained by Kanno (1963) who found that in two groups of *A. broughtoni* larvae, 33% and 10% survived to attachment, and further went on to record the rate of growth following attachment when the spat were maintained in laboratory tanks. These data suggested that attached spat grew from 0.28 mm to 8.21 mm in one year.

Ting et al (1972) achieved some success in inducing spawning of *A. subcrenata* using such treatments as: 1) thermal shock, 2) immersion of

female spawners in a sperm-sea water suspension, and 3) immersion in dilute ammonium hydroxide. To induce spawning by thermal shock, spawners which had been maintained at 27°C were transferred to water at 20°C (time not specified) then back to 27°C. Thereafter the water temperature was raised to 30°C. Using this treatment a response in which 50-60% of blood clams spawned was obtained. When immersed in sperm-sea water suspension, a 62% response was achieved. Responses of 20-64% were acquired by immersion in a weak solution (0.001-0.005N) of ammonium hydroxide in sea water. Responses of 46% in female and 76% in male spawners occurred when the body cavity was injected directly using a 0.01N solution of ammonium hydroxide.

Wong et al (1985) have reported some success in artificially inducing spawning in *A. granosa.* They induced spawning by alternate immersion of spawners in seawater at 16-18°C and 30-32°C. Spawning took place after the second exposure to the higher temperature with the release of mature eggs and sperm. Cell division started 10-15 min after fertilisation and active trochopore larvae appeared after approximately 4 hours. The D-shaped larval stage was attained in about 22 hours. When the larvae were maintained at a temperature of 26-30°C and fed on a diet of *Isochrysis sp.*, they reached the umbo stage after 13 days and settled after 21-22 days. Shell length at settlement ranged from 230-250μm and the larvae metamorphosed into the adult form 28-30 days following fertilisation. Following settlement, spat were fed on a culture of mixed brown algae and attained shell lengths of 2.5 mm after 28-30 days.

To some extent, problems of pollution are peripheral to the main focus of this study which is the reproduction and growth of *A. granosa* and *A.*

antiquata. However, the potential hazard to both natural populations and mariculture activities may be affected by local water quality and the extent of contamination by anthropogenic material is clearly important in this regard. Pesticides and other synthetic chemicals can, even in low concentrations, drastically affect the physiology of fish and shellfish, with which the species may have had no previous evolutionary experience. Pollutants pose a threat for at least two reasons: they may have a directly toxic effect on the organism itself, and they may by virtue of contamination of the organism, render it unsafe for human consumption.

Information relating to the direct toxic effects of either organochlorine pesticides or heavy metals or hydrocarbons on any of the life stages of *A. granosa* is limited, and so far, none are available for *A. antiquata*. Several studies on the average values of a range of heavy metals, e.g . Cd, Cu, Hg, Pb and Zn in the soft tissues of marketplace samples of *A. granosa* harvested in Malaysia and Thailand, show that none of the levels found were particularly high (Phillips & Muttarasin, 1985), with the exception that Huschenbeth and Harms (1975) detected slightly elevated concentrations of Cu (5.6 $\mu g.g^{-1}$). The range for several heavy metals were Cd 0.28-1.91$\mu g.\ g^{-1}$, Cu: 0.51-1.63 $\mu g.g^{-1}$, Hg: 0.01-0.02 $\mu g.g^{-1}$, Pb: 0.13-0.46 $\mu g.g^{-1}$ and Zn from 6.2-19.9 μ $g.g^{-1}$ (Phillips and Muttarasin, 1985). Indeed, these authors concluded that none of the samples analysed contained levels of trace elements which would indicate the existence of potential public health problems. Since the results are based on single samples, more information about intersample and interlocation variability is required before definite conclusions can be drawn. Although the results give no cause for concern that anadarinids may show a particular tendency to accumulate trace metals, this should not be an excuse

for complacency, because another study on *A. granosa* in Semarang waters Indonesia (Anwar et al, 1990b) reported that both soft tissue and the shell of the blood clams did indeed accumulate trace metals at high levels. The average values of Cd, Cu, Cr, Hg, Pb and Zn were comparable to those studies carried out in Thailand, Malaysia, Korea, Japan, Hong Kong, Australia, Mexico and India (Phillips & Muttarasin, 1985). Moreover, Anwar et al (1990b) recorded that the concentration factors for *A. granosa* ranged from 403-1413 for Cd, Cu: 18-108, Cr:11-31, Hg : 8-31, Pb: 19-108 and for Zn it is 15-66 times and that these figures appeared to be site-specific. The concentration factors calculate the magnification of those metals concentrated in the tissues compared to that dissolved in the water.

Information on organochlorine residues in *A. granosa* is limited to two studies. In one single sample of *A. granosa* from the Phuket area of Thailand Huschenbeth and Harms (1975 cited in Broom, 1985) detected the presence of dieldrine, DDT derivatives, lindane and PCBs (polychlorinated biphenyl compounds) at concentrations as high as 9$\mu g.kg^{-1}$, 35$\mu g.kg^{-1}$, 4$\mu g.kg^{-1}$ and 31$\mu g.kg^{-1}$ wet tissue weight respectively. Jothy et al (1983) found an average of 3 $\mu g.kg^{-1}$, 35.5$\mu g.kg^{-1}$ and 33.3 $\mu g.kg^{-1}$ wet tissue weight of dieldrine DDT derivatives, and PCBs from six samples of *A. granosa* from Penang and Perak in Malaysia. Although the levels for PCBs were similar in samples from these two areas, the level of DDT derivatives are 3 times higher in blood clams in Thailand.

Marine organisms in general, and lamellibranchs in particular, are known to accumulate many polyaromatic hydrocarbons (PAHs) that are present in ambient waters, even at levels of parts per billion (Neff, 1979). The toxic effects of these PAHs in seawater increase with molecular size of

the compound-attributed to diaromatics characterised by naphthalene and alkyl naphthalene (Anderson et al, 1974; Abel, 1976; Neff et al, 1976; Stainken, 1978). The physiological and biochemical basis of naphthalene (Nap) intoxication in *A. granosa* from Bombay waters was carried out under laboratory and environmental conditions (Patel et al, 1985; Eapen and Patel, 1989; Patel and Eapen, 1989a, b). The results showed that behaviourally, clams exposed to high levels of Nap (10-15μg/ml, for 96 h) exhibited characteristic movements of the shell valves and foot; the shell valves opened widely within a few hours and the muscular foot was stretched vertically upwards with copious secretion of mucus. Clams did not exhibit any evidence of burrowing behaviour and on transfer to a stressor-free medium, they became intoxicated and narcotised.

Their studies further showed that bioaccumulation of Nap increased over the first 9 hours and then gradually decreased. Tissue distribution of Nap after 9 hours exposure at 10μg Nap.ml^{-1} showed the highest bioaccumulation in the gills, followed by the digestive gland, adductor muscles and mantle folds including the foot and blood corpuscles. The equilibrium between internal tissue compartments and the external ambient level was established within a fairly short period of 2 to 3 hours. Conversely, on transfer to Nap-free medium, clams depurated a major portion (>60%) of the total body burden within the first 3 hours, irrespective of earlier exposure. However, induction of anaerobiosis and disruption of osmotic balance on exposure to Nap, together with aerial exposure at low water periods and salinity changes, acted synergistically and proved detrimental (Patel and Eapen, 1989a, b).

The mechanism of transfer of PAHs from water to organism, like that of trace metals and radionuclides, is controlled and facilitated by an intrinsic liquid/water partition coefficient through biological membranes (Simkiss et al, 1982). The food and feeding habit of anadarinids is likely to increase the potential risk. In the natural environment, a great deal of particulate matter enters the mantle cavity of anadarinids, and since some trace metals associate preferentially with particulate material by surface adsorption (Phillips, 1980), anadarinids are especially vulnerable to such pollutants.

Reports in the literature of the likely sources of nutrition of both *A. granosa* and *A. antiquata* are still almost non-existent. So far, it is generally accepted that bivalves other than septibranchs are specialised herbivores whose guts rarely contain faunistic material (Owen, 1966). However, Broom (1982b) found that benthic diatoms and foraminiferans examined in water collected from the benthic layer, are invariably found in the gut of *A. granosa*. A dense population of *A. granosa,* a facultative surface deposit feeder, may significantly reduce the amount of chlorophyll in the benthic boundary layer, compared with the quantity of chlorophyll in the benthic layer of the adjacent mudflats where no such population is present. Moreover, the only information for *A. antiquata* is that from Kasigwa and Mahika (1991) working along the Dar es Salaam coast of Tanzania. Their results indicate a wide phytophagous mode of feeding. Apparently the clams ingest 27 genera, with well over 30 species, of phytoplankton on just one beach alone. Members of classes Bacillariophyceae, Chlorophyceae, Pyrophyceae and Prasinophyceae were very prevalent in the diet.

In general, bivalve molluscs are not particularly well adapted to filtering water with high concentrations of suspended solids. Consequently, unless

they are specifically adapted for deposit feeding (e.g. tellinids, nuculanids) they tend to be excluded from soft mud substrata where there may be substantial resuspension of particulates (Rhoads and Young, 1970). Members of the Anadarinae lack well-developed siphons. Openings of the inhalant and exhalant streams, formed from folds in the posterior mantle edge, lie flush with the posterior margin of the shell and do not extend beyond it. As a result, most species do not burrow into the mud to any depth.

Many of the anadarinids, however, appear to have become adapted to exploit the niche of filter feeder. They can cope with high concentrations of suspended solids as recorded by Broom (1982b) for *A. granosa* and Lim (1966) for three other species of *Anadara*. Lim (1966) compared the ciliary currents in *A. antiquata*, *A. anomala* and *A. cuneata* which live in environments where they experience markedly different concentrations of suspended solids. *A. antiquata* inhabits rocky areas, *A. anomala* occurs in sandy substrates, whilst *A. cuneata* establishes deep burrows in relatively firm mud. Lim (1966) found that the labial palps of *A. antiquata* have 40 transverse folds, those of *A. anomala* 43, those of *A. cuneata* 150. The order of complexity of their labial palps was *A. antiquata*<*A. anomala*<*A. granosa*<*A. cuneata*, suggesting that this was probably due to differences in the environment occupied by these four species with *A. cuneata* having to deal with the heaviest load of suspended material. Furthermore, it was also noted that in both *A. cuneata* and *A. granosa* there is a strong ciliary current carrying particles collected from the outer surface of the labial palps onto the inner surface of the distal portion of the palps. This was not present in either *A. anomala* and *A. antiquata*.

Yoloye (1975), working on *A. senilis* reported ciliary currents similar to those found by Lim (1966). Strong ciliary currents on the visceral mass, foot, and mantle, removed silt and sand from the mantle cavity and prevented clogging of the gills. It was also noted that *A. senilis* possesses particularly well-developed labial palps of considerable length (4mm) and that these play a major role in sorting large quantities of particulates.

Several contributions concerning various aspects of the biology, ecophysiology, biochemical properties, culture, as well as fisheries aspects of *A. granosa* and *A. antiquata* have been published elsewhere (Pathansali and Soong, 1958; Pathansali, 1963, 1964; Broom, 1980, 1981, 1982a,b,c, 1983b, c, 1985; Boonruang & Janekarn, 1983; Davenport & Wong, 1986; Nair, 1986; Ng, 1986; Kayombo & Mainoya, 1987; Richardson, 1987; Patel & Eapen, 1989a,b; Kasigwa & Mahika, 1991). However, almost none of these studies relate to the populations in Indonesia waters, except for those by Ismail (1971), Sudradjat (1978) for *A. granosa* and Kastoro (1978) for *A. antiquata* respectively.

Considering that both of these species, but in particular *A. granosa*, are important economically as food and as potential biomonitors of coastal water quality (Patel et al, 1985; Phillips & Muttarasin, 1985; Anwar et al, 1990; Eapen & Patel 1989), as well as being ecologically dominant members of many shallow water communities (Broom, 1983c), there is clearly a need to study the ecological aspects of these species in Indonesian waters. This study concentrates principally on the growth and reproductive cycles of *A. granosa* which live mainly in muddy substrata and *A. antiquata* which occurs predominantly in sandy sediments.

The first chapter deals with general introduction of the anadarinids, in particular *A. granosa* and *A. antiquata*. Chapter two describes site and environmental characteristics of the three study sites, sampling technique used during field work, population density, pattern of dispersion and associated macrofauna in the blood clam beds. One species studied, *A. granosa* is known to partially burrow in marginally subtidal to subtidal areas in muddy substrata seaward of mangrove stands, whilst the other, *A. antiquata*, is a poor burrower recorded in the intertidal sandy areas of coral reef-associated habitats. Chapter three details the reproduction of the blood clams *A. granosa* and *A. antiquata* through qualitative changes in histological preparations of the reproductive tissues, quantitative measurement of sex ratio, density and size of the oocytes, assessment of changes in the dry tissue weight as well as condition index of these two blood clams, and briefly describes pea crab parasitism on *A. antiquata*; besides providing evidence of sequential protandric hermaphroditism. Chapter four is concerned with allometric and absolute growth, the latter assessed from changes in monthly population structure, and microgrowth patterns in particular of *A. granosa*.

Each aspect of the subject matter of this study is introduced and discussed separately in the individual chapters and, a general discussion at the end attempts to synthesise the information gained on the reproduction and growth of these congeneric species, comparing and contrasting such patterns in relation to the range of habitat studied.

CHAPTER II

STUDY SITES AND ENVIRONMENTAL CHARACTERISTICS

2.1. Introduction

Both *A. granosa* and *A. antiquata* appear to occupy areas with an estuarine influence, but their preference of sediment grain size and position in the shore are different. In general, the population attributes of shallow water molluscan species can be related to many environmental factors, including food supply, salinity, temperature, oxygen availability, water movement and turbidity, substratum composition and physical stability of the bottom, and concentration of chemical pollutants (Kinne, 1970; Sindermann, 1979).

Estuaries are usually considered as difficult environments where physical conditions fluctuate widely and are not rigidly predictable. The temperature regimes within estuaries are often more variable than adjacent waters due to their shallow nature which exposes the water to both heating and cooling, and also due to the varied input of water at different temperatures (McLusky, 1989). Besides the more saturated fresh water that flows into estuaries, additional oxygen will be supplied through the water surface and by plant photosynthesis - the turbidity of estuarine waters may limit phytoplankton production by restricting light penetration. However, the organisms living within estuaries, especially within the bottom deposits, rapidly consume oxygen and thus many sediments may become anoxic,

except for the very thin layer on the surface (Boaden & Seed, 1985). Where excessive organic enrichment occurs, the multiplicity of microorganisms so produced may also consume all the oxygen within the water body (Meadows & Campbell, 1987).

In most estuaries there is a close relationship between salinity and substratum type with reduced salinity associated with finer sediments. This phenomenon occurs because clay particles (0.12-1.95μm) tend to adhere to each other. As 'salt flocculation' occurs particles will tend to fall faster, and even a few parts per thousand of salt is sufficient to make suspended particles cohesive (McLusky, 1989). This flocculation, which is a reversible phenomenon, begins at about 4‰ salinity, is increased by high temperatures and also by carbohydrates in solution, and decreased by the presence of proteins (Meadows & Campbell, 1987).

Salinity determines the distance that a species is capable of penetrating into an estuary, but the full potential of any species which enters the estuary can only express itself when suitable sediments are present. For example, high turbidity which is associated with physically unstable fine grained sediments, is detrimental to suspension feeders as it enhances the real problems of gill clogging and sediment removal, those taxa are therefore excluded from such habitats (Rhoads and Young, 1970; Morton, 1983a). The change from an aerated surface sediment layer to deeper anoxic layers takes place closer to the surface in fine-grained muds than in coarse-grained sands. This change serves to limit the macrofauna in estuarine sediments to species which can form burrows or have other mechanisms to obtain their oxygen from the overlying water.

Belonging to the clam group in the fisheries statistics (FAO, 1991), arkshells are among the most widely distributed and utilised marine shellfish and their culture is second in antiquity only to that of oysters among aquatic invertebrates (Bardach et al, 1972). Clam culture however, has never become as widespread or highly developed as for instance oysters or penaeid shrimp. This perhaps because clams are so abundant and easy to harvest in nature.

As a group, *Anadara spp* contribute a substantial amount to commercial landings relative to other marine molluscs. In Indonesia, commercial catch on *Anadara spp* which mainly consists of *A. granosa,* is second to that of penaeid prawn, whereas common squid and jellyfishes were third and fourth in importance. The nominal catches for *A. granosa* in Indonesian waters were increased from 30.289mt (metric tons) in 1986 to 35.150mt in 1991. Whilst in the worldwide, squid, cuttlefish and octopuses were the highest marine shellfish group landed ($2560x10^3$mt), the second was clams, cockles and arkshells ($1540x10^3$mt), followed by mussels ($1332x10^3$mt) and oysters ($1007x10^3$mt; FAO, 1991).

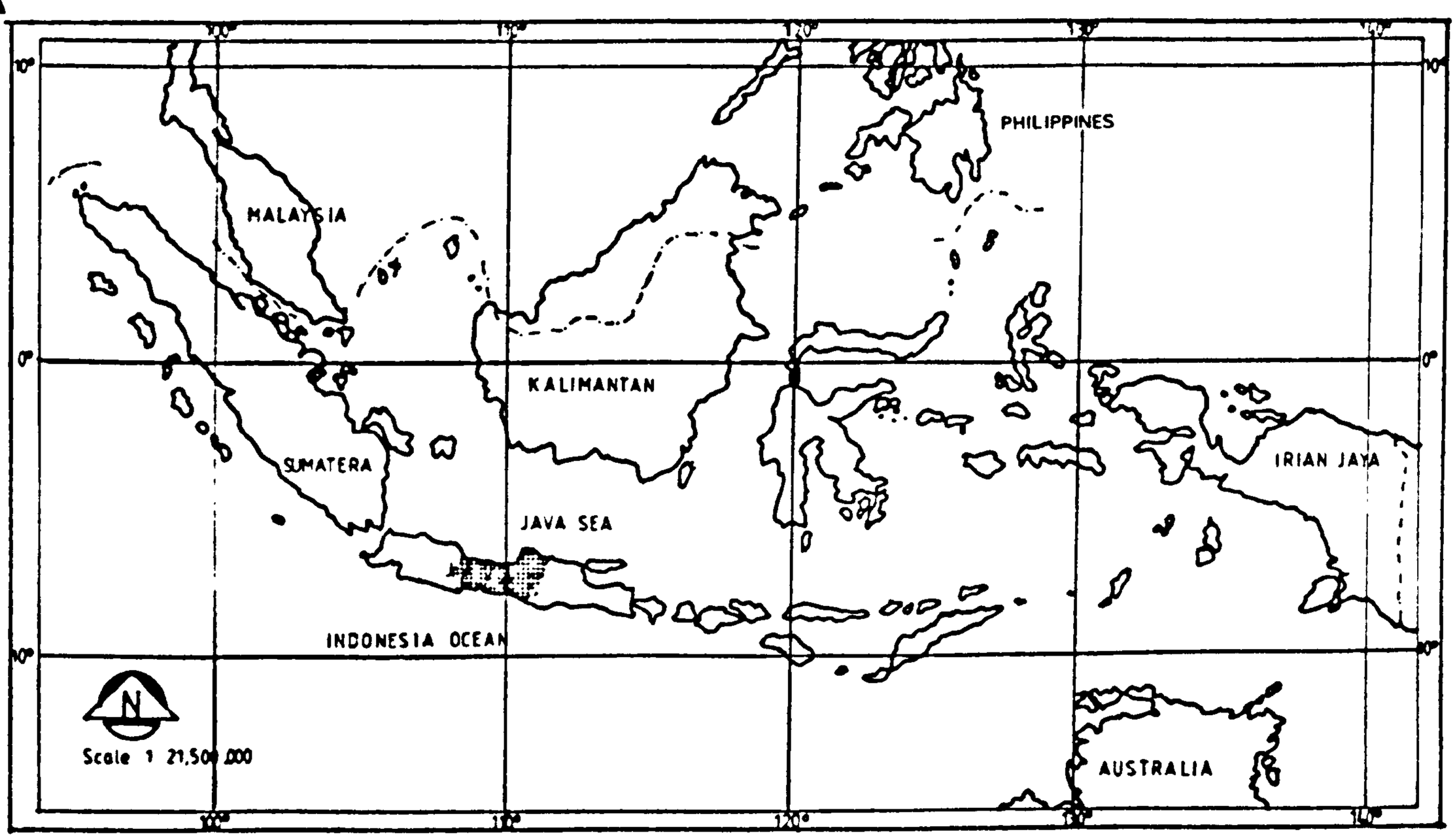
The populations of *A. granosa* in Malaysia which have been cultured practically since 1948 and have been described by a number of authors are well summarised in Broom (1985). Furthermore, Malaysia applied a strict government regulation upon the legal marketable size of *A. granosa* as well as the minimum size of spat collected for cultivation. This species which has not been cultured extensively in Indonesia and yet the landings were comparable to those from Malaysian waters (see FAO, 1991), has received a little attention in regard of its basic ecology. The previous studies of *Anadara* in Indonesia have been concerned more with their fisheries status (Ismail, 1971; Kastoro, 1978; Sudradjat, 1978).

This chapter briefly describes the sampling techniques used during field work, the population density and pattern of dispersion of the study populations, notes pea crab infestation in *A. antiquata* and the associated macrofauna within the *Anadara* community at the three study sites. Since the distribution of sediments and salinity within estuaries, along with the distribution of oxygen, temperature and phytoplankton availability are all interdependent, this chapter also attempts to interpret the interrelationships between these environmental variables and how these affect the populations of both the species studied.

All the field work and some of the laboratory analyses of this study were conducted in Indonesia. The climate of Central Java is equatorial with small daily and seasonal temperature variations. The average daily air temperature during the study period was about 32°C with a seasonal range of 4°C and a mean daily range of 3°C (Balai Pengawasan Tanaman Pertanian, 1993). Tides in the study areas are mixed semidiurnal but prevailing diurnal pattern with spring tides occurring twice a month. The locations studied, i.e. Wedung, Tapak and Bandengan, are shown in Figure 1 with details of each site in Figures 2, 3, 4 and Plates 1,2,3.

Whilst Wedung supports one of the biggest commercial fisheries for *Anadara granosa* in Central Java, the natural population of *A. granosa* in Tapak is relatively undisturbed and commercially unexploited. At Bandengan, local people harvest *A. antiquata* on a subsistence basis. For *A. granosa*, each site was purposely selected in order that comparisons of the environmental parameters studied could be made between disturbed (Wedung) and relatively undisturbed (Tapak) populations. The third site at Bandengan was chosen to allow a comparative study of the allometric

A



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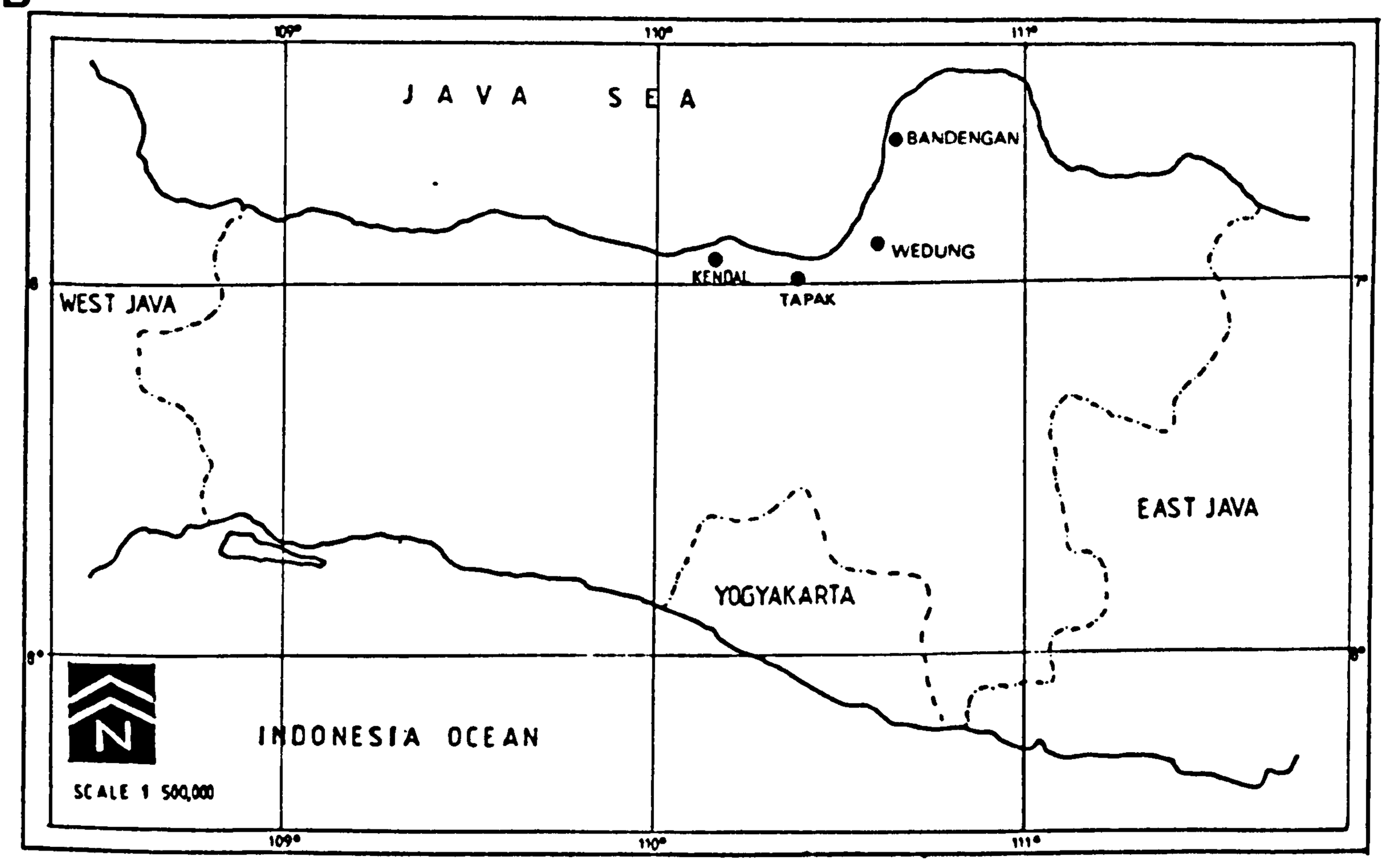


Figure 1: A. Map of Indonesia and B. Map of Central Java

relationships and reproductive cyclicity within this congener. These three sites are relatively close to, and accessible, from Semarang (07°0S; 110°4'E) where the base laboratory was located.

2.1.1. Brief descriptions of study sites

2.1.1.1. Wedung

The town of Wedung is located about 50 km north-east of Semarang (06° 45'S; 110°35'E), and a journey of at least 1.5 hours down the river Wulan at high water in a motorised shallow-draft wooden boat 6 m long was needed to reach the sampling site (Fig. 2, Plates 1A and C). The area is a semi-sheltered water body within a large bay, with several large rivers (Tuntang, Wulan and Serang) and many other smaller ones (Plate 1B) opening into the bay thus resulting in considerable local deposits of mud and high turbidity. The site is protected by a mud flat which formed in 1947 and has been widening ever since. The bottom sediment is composed of fine mud and compared with Tapak is relatively free from the small turret gastropod *Cerithidea cingulata*. The area is surrounded by a dense mangrove swamp forest consisting mainly of *Avicennia marina* and *Rhizophora apiculata*.

At Wedung, local fishermen have divided the collecting area for *A. granosa* into two. The low intertidal and marginally subtidal areas are fished manually, whilst the area nearer to the open sea requires the use of fishing gear attached to a motorboat (Plate 1C). Collection of blood clams during the period of this study mainly occurred from June to January but ceased in July 1992 when the fishermen left to fish in another nearby area, Kendal. Peak of clam collection took place during August-November. In September 1992 most of the fishermen again returned to Kendal to fish for *Scapharca*

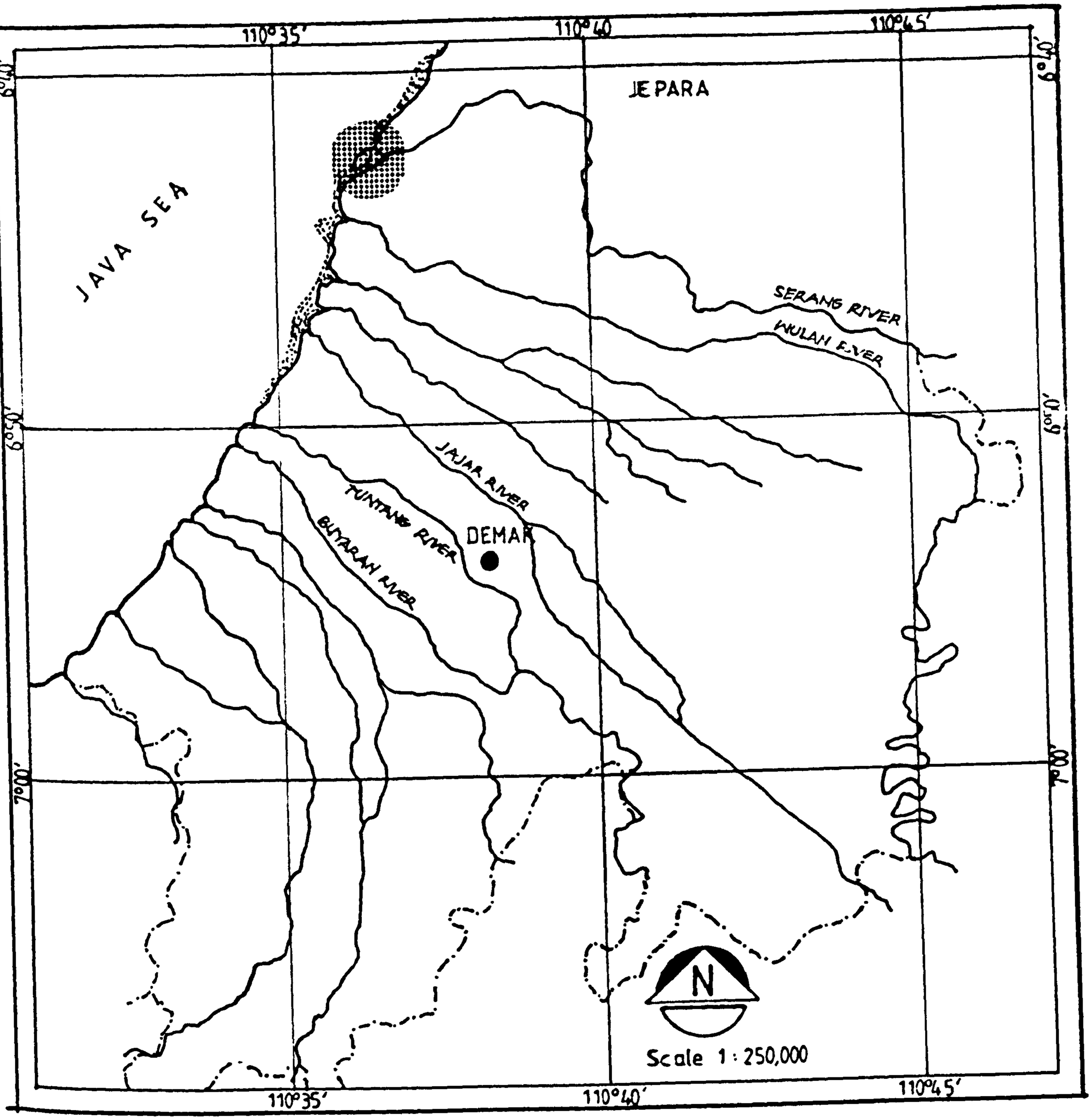


Figure 2: Map of the study site in Wedung

Plate 1: Photographs of the study area in Wedung

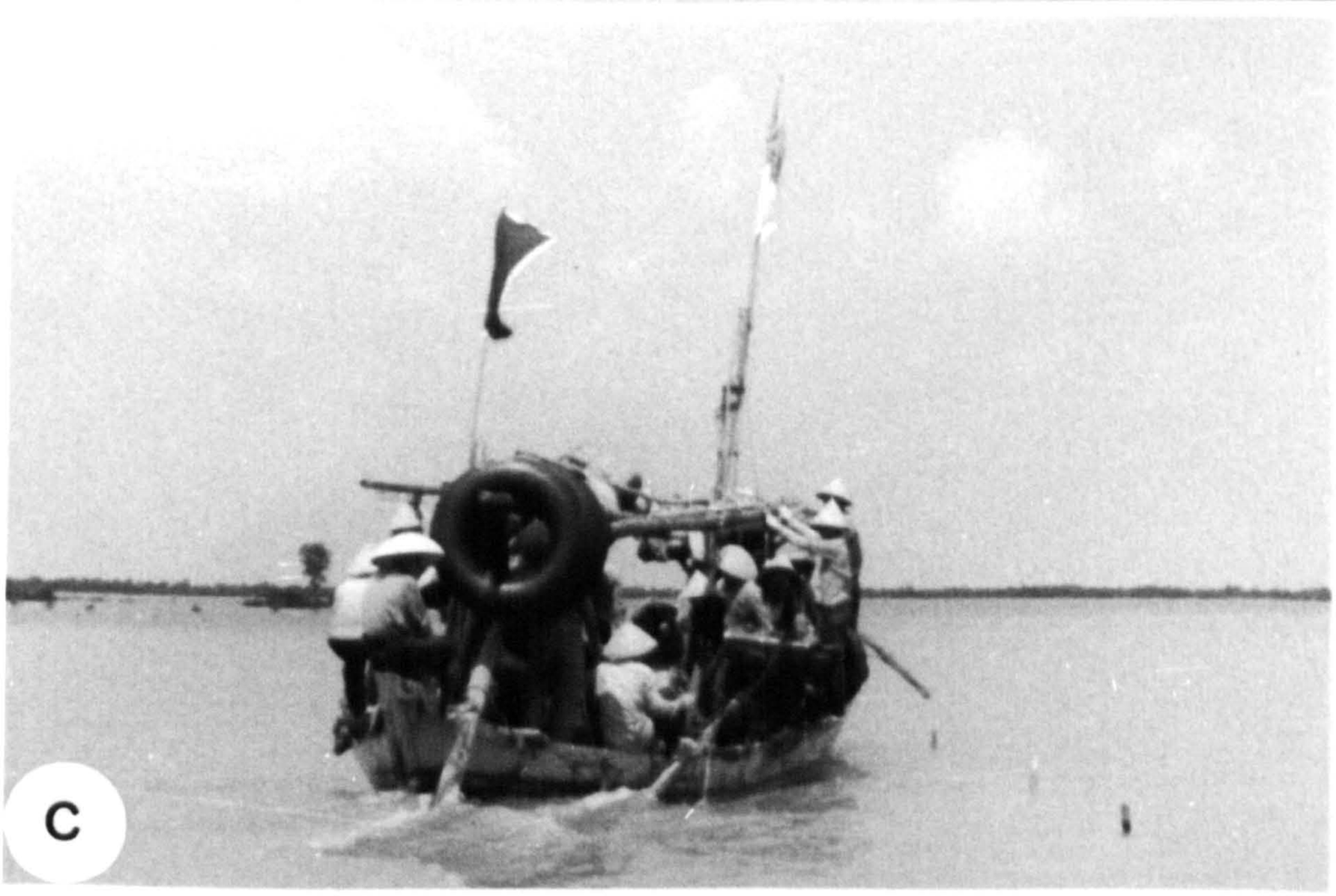
A. Mangrove stands bordering the flat and sheltered site.

B. One of the small rivers opening into the bay, note the thick mangroves lining the river bank.

C. A fishing boat normally with 10-15 fishermen used to trawl the blood clams subtidally using a traditional fishing trawl. During low tide or in shallow water they collect the clams manually using the inner tubes shown on the boat to float their collecting baskets. Three bamboo poles seen on the right of the fishing boat mark the location of the experimental plots during low tide.


A


B


C

cornea, another anadarinid, which they landed in considerable quantity in Wedung. After a big flood in December 1992 most fishermen reverted to trawling for prawn and fish in the open sea, whilst only a few manual collectors remained to gather any remaining clams that were present at a much reduced density.

Although there is a fish market in Wedung run by the Department of Agriculture, this is mainly for prawns. Blood clams, which may be landed in considerable amounts, are not registered and therefore no records are kept for they are always sold directly from the fishermen to the local buyer who collects them for another buyer in the marketing chain.

2.1.1.2. Tapak

The village of Tapak is situated in a large bay some 25 km north-west of Semarang. This study site is a small sheltered shore adjacent to the mouth of the river Karanganyar where a sandbar (the island of Tirang Cawang, Fig. 3 and Plate 2A) effectively protects the shoreline from the open sea. Besides two large rivers at its eastward end, which carry all the sewage from the city of Semarang, three other small rivers also discharge into the bay. Of these, the river Tapak, which has a length of 2.5 km and runs through the middle of wide areas of paddy fields and intensive milkfish (*Chanos chanos*) tambaks, discharges heavy loads of organic and inorganic pollutants from a big industrial area (43 manufacturers) further upstream. The industrial area was established some 15 years ago and during the last 4 years, when this study had just begun, became a national issue regarding the discharge of untreated pollutants. In 1992, the Government and local businessmen paid financial compensations to the local fishermen, particularly those owning tambaks.

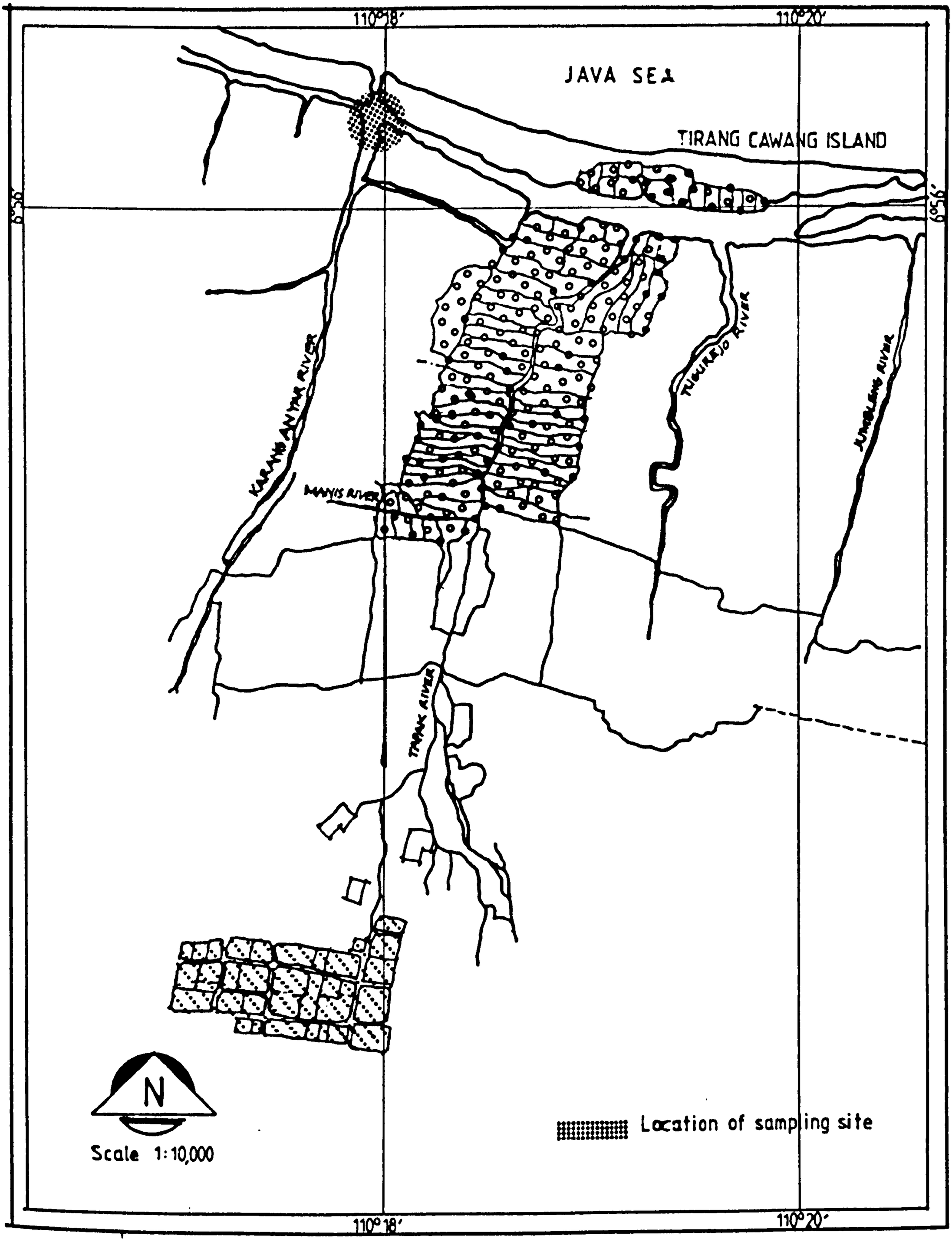


Figure 3: Map of the study site in Tapak
▨ is the industrial areas and ⊡ is the milkfish tambaks.

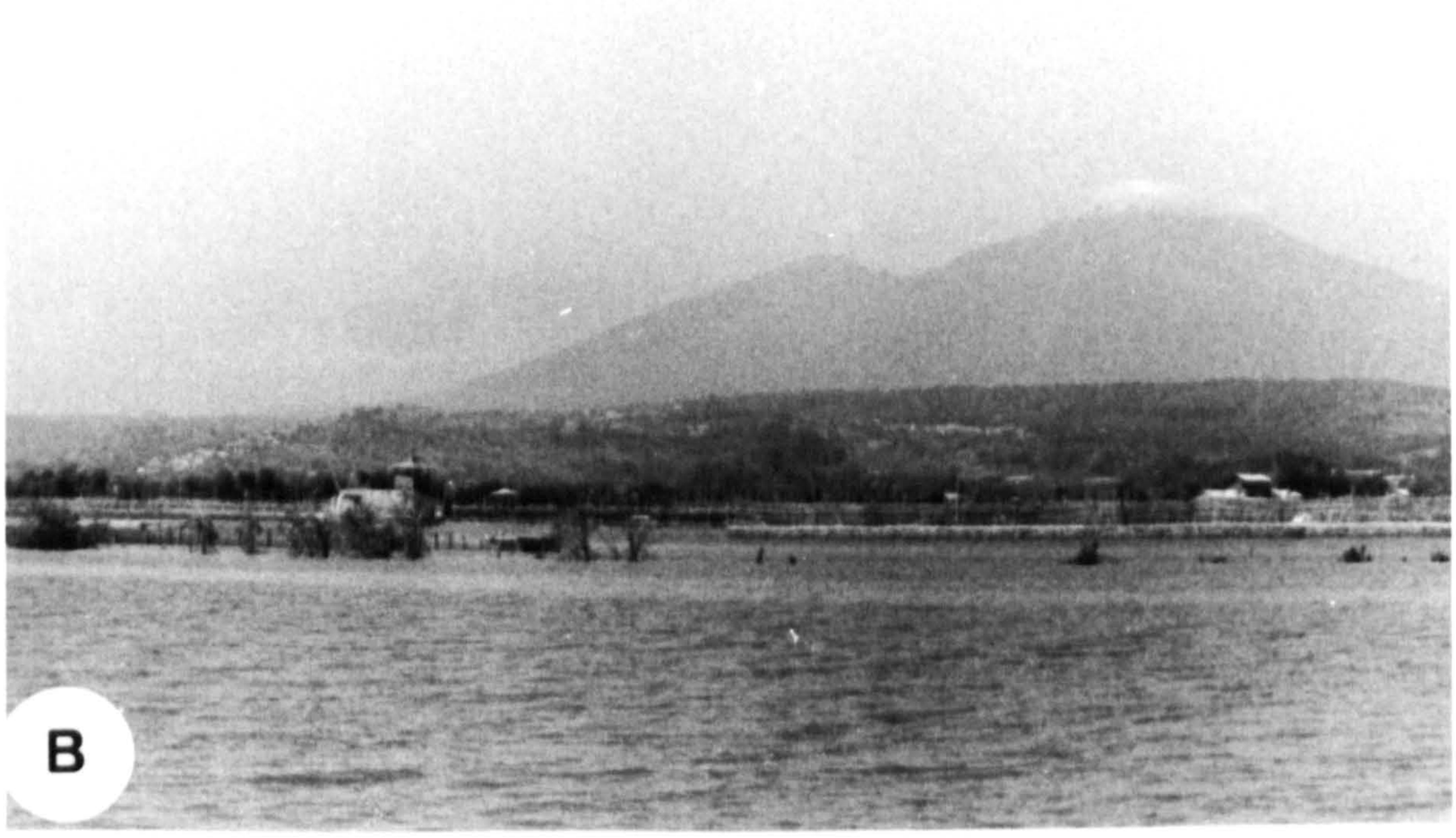
Plate 2: The study site in Tapak photographed at low tide.

A. The sandbar island of Tirang Cawang, protecting sampling areas from the open sea.

B. A concrete wall of a prawn culture pond seen in the distance bordered the landward margin of the sampling areas.

C. An abandoned tambak used as a sampling area in Tapak. A bambo stake seen in the foreground on the left marked the plot.


A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The area under study, which is about 2 km^2, relatively flat and shallow (4 m maximum depth at high water of spring tides), and always turbid. The bottom sediment is muddy (30-40cm). During the period of this study, at extreme low water of spring tide during January 1992 and 1993 the area almost completely drained out. The landward margin of this study site is bordered by the concrete wall of a prawn culture pond (Plate 2B). A sporadic fringe of mangrove trees *Avicennia marina, Kandelia kandel* and *Rhizophora apiculata* is present in the middle of the area which was previously used as a tambak (Plate 2C), whilst *Pandanus tectorius* together with some *A. marina* and *R. apiculata* form a dense fringe around the island of Tirang Cawang (Plate 2A). At this site, local people fish for prawns, oysters (Ostreoidea), finfish, or in the tambaks themselves for the edible crab *Scylla serrata.* Throughout the study period, the bottom sediment was always covered by a dense population of *Cerithidea cingulata.*

2.1.1.3. Bandengan

This study site is situated in a relatively open sandy shore 115 km north-east of Semarang (6°33′07″S; 110°40′E), which also serves as a recreation beach. The shore is relatively flat with an average water depth during low tide of 0.5m and is characterised by sandy sediment mixed with boulder, gravel and coral rubble (Plates 3A and C). The water is relatively clear compared with the sites at Tapak and Wedung. Dense stands of *Pandanus tectorius, Saccharum arundinaceum*, and *Scaevola cericea* border the shore line (Fig. 4 and Plate 3A). On this beach, local people, usually women and children, collect clams on a subsistence basis for their own consumption or selling to visitors. Some illegal activities of coral digging

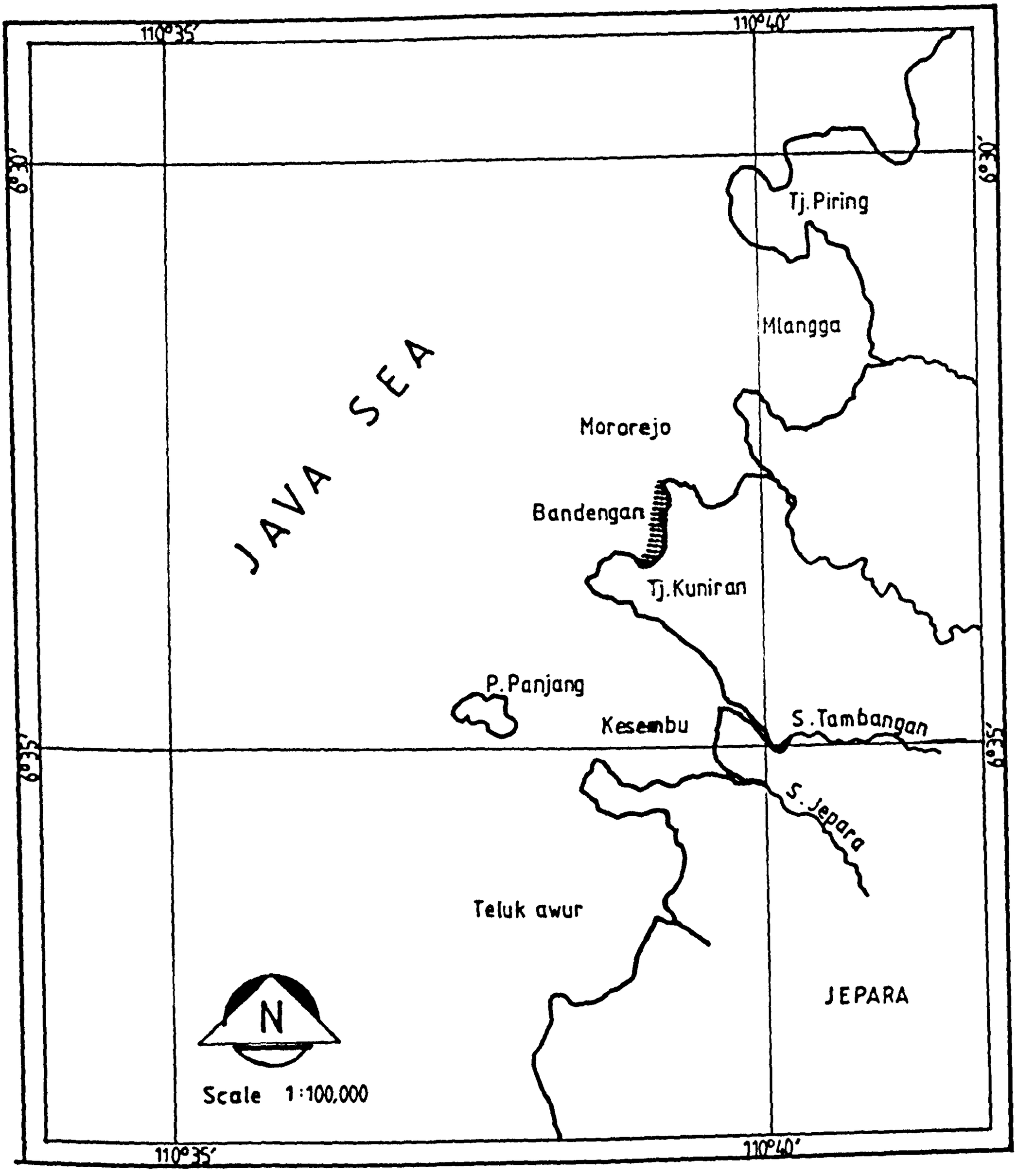


Figure 4: Map of the study site in Bandengan

Plate 3: The study site in Bandengan photographed at low tide

A. Dense mangrove stands bordering the intertidal sandy area at low water of neap tides.

B. The tidal flat that may extend for over more than 500m during low water of spring tides. This part of the beach is popular for recreational use.

C. A pool left by the activities of illegal coral digging. The blood clam *A. antiquata* occurs in such pool providing the pool does not drain out during low tides.

A

B

C

altered the shoreline profile and left it with a number of pools (Plate 3C) in the low tide shore. The tidal flat (0.2-1m depth) extends over a distance of 500 m during low water of spring tides (Plate 3B).

2.2. Materials and Methods

2.2.1. Field work

The use of bamboo stakes to mark the sampling positions on the shore was often not practicable because of interference from local people. Therefore in order to obtain a position fix for the sampling area, at least three prominent landmarks were noted. Errors were minimised by using a compass contained within a telescopic lens. In addition, rough sketches of the general area together with photographic records of the main features of the local environment facilitated the precise relocation of each site. Sampling of the blood clam populations was conducted at approximately monthly intervals at low water during the hours of daylight over 24 months between August 1991-August 1993. At each site, quantitative random samples were obtained from within a marginally subtidal area with a maximum depth of water of 1 m at low tide in Wedung and Tapak, or 10-20cm in Bandengan.

In Wedung and Tapak, even at low water of spring tides, the clam beds were completely submerged and access was impossible without the use of a boat. The depth of mud at both sites was 30 - 40 cm which made it difficult to walk. Since there appeared to be no difference in bottom slope within the mid-shore at Wedung, three plots, each 7m in radius (ca.154 m^2) and separated by 50 m, were established approximately 200 m from the shore line. These plots, which were established more or less in the central area of

the clam populations, were unfortunately located on the route frequently used by local motorboats. Therefore the plots could only be marked permanently by placing a bamboo stake approximately in the middle of each plot. However, these stakes were often lost, in which case they were quickly replaced. Although the exact zonal status of each plot was not established, it is unlikely that any slight differences in tidal position would be a major factor influencing the results obtained between plots. Sample collection in Bandengan was normally taken following a transect parallel to the shoreline in the mid-shore area (10-20cm water depth) during low tide.

The method of sampling at Wedung and Tapak involved tying a 7m length of rope marked with 1m divisions to the centre pole, then on hands and knees, crawling around the plot marked by the traverse of the rope about the centre pole and collecting all the animals encountered within the circular area. Due to the amount of daylight available in one tidal cycle (8 hours), the clarity of the water column (0.2-0.4m depth using a Secchi disk), and the patchiness/ scarcity of the specimens, this method of sampling an area of ca. 150m^2 was preferred to that of using a quadrat. However, a few quantitative samples for estimating population density and the dispersion pattern were collected using a quadrat.

Quadrats of 1m^2 were applied on 5 separate occasions in Wedung. Some of which coincided with time when the spat seemed to be approaching their peak abundance, i.e. August 1991, August-September 1992 and July-August 1993. Except for the occasions specified above, samples for estimating densities were taken intermittently. This was because of the progressive reduction in the density of the population during the period of

study and the difficulties encountered with the one-man operation of the cumbersome Ekman grab sampler.

On these occasions, between 10-15 quadrats were collected randomly from within the circular sampling plots in order to estimate the density. In Wedung, most of the blood clam collections were not sieved because some samples had an admixture of stiff clay, for the bottom sediment appeared to be compacted as a result of manual collection of blood clams by local fishermen. As a result, quadrat samples which were also collected for sediment particle size analysis, were searched manually instead of sieving. Indeed, as sieving was not attempted, spats were not likely to be found. Because blood clams were sparsely distributed at Tapak, several of the quadrats samples contained no specimens. Therefore estimates of the density of the population of *A. granosa* in Wedung and *A. antiquata* in Bandengan were made, but not for the Tapak population. In the subsequent analysis, all blood clam density estimates were related to an area of $1m^2$.

In Bandengan, sample collection involved digging out a soil profile within the quadrat using a shovel, raking and then searching the sediment for *A. antiquata*. The larger clams were picked by hand whilst the remaining sandy mud was sieved through a mesh size of 5mm and rigorously shaken with ample additions of sea water, thereby retaining small clams on the sieve. However, this technique rarely gave an adequate number of individuals for analysis compared to the alternative methods of collecting samples randomly from mid-shore areas when a water depth of 0.5-1m depth covered the sediment. Sieving was carried out only on a limited number of occasions (September 1991, January, June, November 1992, January and July 1993) to estimate population density. In order to collect *A. antiquata* from the mid-

shore areas, the water overlying the blood clams needed to be clear and transparent so as to locate the position of the burrows traced on the surface by the small whitish 'siphon-like' holes adjacent to each other.

Since *A. antiquata* inhabits shallow water areas, it was possible to determine their pattern of distribution relative to the tidal level; quadrat samples of $1m^2$ were taken at intervals of 50m parallel to the shoreline from the water mark at low tide in June 1992. The differences in sampling technique applied in Wedung, Tapak and Bandengan were mainly due to the different local conditions, e.g. location of the sampling areas relative to the shore line/water depth and nature of the bottom sediment.

Throughout the period of study, many unsuccessful attempts were made at all three study sites to establish experimental cages for growth rate estimations. Most of the cages were stolen. However, towards the end of the study some successful experiments were carried out and an experimental plot was maintained for 119 days (7 February - 5 June 1993) in Bandengan and another one for 64 days (5 June-8 August 1993) in Wedung. Both of these were in the vicinity of the plots which were routinely sampled but in areas rarely visited by local fishermen. In this study, an attempt was undertaken to transplant spat of 12-16mm length from Wedung to Tapak (August 1992 and April 1993). However, the reverse was not possible because samples collected from Tapak were normally not even ample for a complete monthly sample analysis, so no spare specimens were available for transplantation.

Clams were marked by making a slight notch at the posterior ventral margin of their shells using a fine metal file, so as not to damage the mantle edge. Notching of the shell edge was designed to interrupt the normal pattern

of shell deposition to which all further growth could be related (Richardson, 1993).

An initial transplant experiment was conducted in Tapak using 250 specimens of *A. granosa* from Wedung; of which 20 file-marked clams were placed into 3 small plastic cages of 40x30x10cm and buried in the sediment. The rest of the blood clams (230 individuals) were sown within a bamboo fence of ca. 1x2m in the main clams bed. This attempt failed due to the loss of both cages and fences, and clams within the fence could not subsequently be located. To avoid being lost again, during a second experiment in Tapak, 5 plastic cages of 40x30x10cm each with 20 marked-animals from a wide size range were submerged in an adjacent tambak from 25 April-14 June 1993. The contents of these cages were sampled after 28 and 49 days in the field.

A similar experimental plot in Bandengan consisted of bamboo stakes arranged as fences, laid approximately at the same distance from the shoreline as the monthly sampling sites but some 500m further south. Approximately 400 file-marked *A. antiquata* of 31-48mm shell length collected on 7 February 1993, were sown in the plot. This size range was selected due to the lack of available juveniles in that particular month. The clams were re-sampled 21, 28, 56, 62, 84 and 118 days later.

The plots in Wedung, however, consisted of 3x3 rows of small unobtrusive bamboo stakes placed at an interval of 10m each at the lower part of the shore, these were emersed only during spring tides but remained immersed during neap tides. The plots were searched prior to the clams being sown, and nothing but *Cerithidea cingulata* was found. Similarly, 300 file-marked juveniles of 18-22mm shell length were scattered onto the sediment

surface around each stake. The experimental animals were recaptured after 14, 36 days respectively and the rest removed after 64 days.

Sediment and sea water temperatures, surface water salinity and pH were monitored *in situ* on every sampling occasion. Salinity was measured using a refractometer, pH on a pH stick renewed every 6 month to maintain accuracy, and temperature by means of a 0.1°C precision mercury thermometer. Water samples for these four physical parameters were collected at the same time and from the same points in the middle of each sampling plot during each monthly sampling occasion. Samples for pH and salinity measurements were taken from the water at 20cm depth. Two thermometers were used to measure temperature. One was hung 20cm below the water surface whilst the other was inserted about 10cm into the sediment. Both were retained in position for 5-10 minutes before any reading was taken. Similarly, sediment samples for measuring the redox potential were collected intact by core and spade in order to approximate the position of the reducing (anoxic) condition in the clams bed. However, the redox probes were easily and often broken and were frequently not available in the store, hence this parameter could not be monitored regularly.

Samples for estimating Dissolved Oxygen (DO) in the water from the sampling plots were taken using 100ml dark BOD bottles. Bottles were rinsed twice with sea water and samples from a depth of 20cm above the sediment were introduced through a long tube into the bottom of the bottle in such a manner as to avoid bubbles while filling. The seawater was allowed to overflow the bottle for 2-3 min before the stopper was inserted immediately. Two reagents, 3M manganous chloride and alkaline iodide (NaOH 8N/Iodide 3M) 1ml each, were instantly added using a syringe pipette, re-inserting the

stopper and the contents of each bottle mixed by inverting. Whilst waiting for other samples to be collected, the bottles were kept in the shade under water. The DO concentrations were determined *in situ* on site within the following 6 hours. Adopting the classical Winkler protocols (Carpenter, 1965; Carritt & Carpenter, 1966; Parsons et al, 1984), one ml of 10N sulphuric acid was added, the stopper replaced and the contents mixed thoroughly until all the precipitate redissolved. After 5 min, all the aliquot of the iodine-containing solution was carefully poured into a beaker glass so as to minimise oxidation through contact with air. It was then titrated using 0.005N sodium thiosulphate in a $10cm^3$ burette. Several drops of starch solution as colour indicator were added when the solution had reached a pale yellow colour, and the titration continued until the blue colour just disappeared. Reagents for analysing dissolved oxygen in the water samples are recorded in Appendix 1.

In order to determine the seasonal variation of food available for reproduction and growth, seston and pigment, quantified as chlorophyll, were assessed. The most useful chemical method for determining the total quantity of phytoplankton in seawater involves estimating the amount of chlorophyll (usually as chlorophyll-*a*). A known volume of seawater is filtered onto a glass fiber filter, extracted in non-polar solvent and the pigment concentration estimated spectrophotometrically (Parsons et al, 1984). For this purpose, one litre samples were obtained by pumping seawater from a depth of approximately 20 cm below the surface into suitable polyethylene containers and analysed on return to the laboratory. All measurements of the above environmental factors and water samples were taken in triplicate and the averages used in data analyses.

In an attempt to correlate particle size diameters to the distribution, population structure and growth performance of the blood clams, between 3-5 cores (PVC of 22.5cm diameter, 30cm long) were taken randomly within each sampling plot. Each core penetrating to a depth of 15 cm, i.e. into the anoxic dark layer. The sediment thus removed was placed in pre-labelled plastic bags. Given the significant effort and time required for subsequent analysis, two cores were considered sufficient for examining between site variation in sediment. No attempt was made to preserve the vertical core structure as the determination of such a gradient was beyond the scope of the present study. All faunal, water and sediment samples were transported back to the laboratory, prior to further analyses.

2.2.2. Laboratory analysis

2.2.2. 1. Pattern of dispersion and the associated macrofauna

In order to asses the degree of aggregation/spatial distribution shown by the two species studied, a simple index of dispersion $Id = s^2/\tilde{N}$ was used, where $\tilde{N}$ is the mean population density estimate (number of indiv.m^{-1}) and s^2 is the variance of that estimate. A coefficient of dispersion of 1 indicates random distribution. Values less than 1 suggest regularity or a discrete pattern, and values greater than 1 indicate patchiness/contagious distribution (Hughes, 1970; Broom, 1982b). Significant departures of the coefficient from 1 are tested using a t-test with (n-1) degree of freedom, where n is the number of observations. The standard error, which is independent of the density and depends only on the number of samples, is given by $\sqrt{2/(n-1)}$.

The occcurence of macrofaunal species associated within the clams bed were sorted, counted and stored in labelled specimen containers containing

10% formalin, prior to their being grouped taxonomically and their density estimated qualitatively.

2.2.2.2. Temperature, salinity, pH, monthly rainfall and tidal regime

Monthly data for surface water and sediment temperatures, surface water salinity, and pH for Wedung, Tapak and Bandengan were noted, averaged and plotted. The nearest town to the study sites was chosen to represent the rainfall conditions during the study, i.e. Demak for Wedung, Semarang for Tapak, and Jepara for Bandengan. The monthly rainfall data were obtained from the Badan Pengawasan Tanaman Pangan Republik Indonesia (1993). The Indonesian Admiralty Tide Tables for Semarang waters 1991-1993 were supplied by the Directorate of Hydro-oceanography, Navy of the Republic of Indonesia, and data from these tables were incorporated into a Turbo Pascal programme provided by Dr. Tony Walne (School of Ocean Sciences, Menai Bridge); a tidal model for the waters around Semarang was generated using fundamental harmonics provided in the tide table.

2.2.2.3. Calculation of oxygen concentration and percentage saturation

Details on the sampling technique used to take water samples for determination of dissolved oxygen were outlined in the field work. The oxygen concentration in water samples ($\mu mole.l^{-1}$) was calculated as follows:

$$DO\ (\mu M.l^{-1}) = \frac{A \times M \times V \times 10^6}{(B \times (V-2) \times 4)}$$

where: A is the volume of thiosulphate added in cm^3, (1 cm^3 of 1M thiosulphate ≈ 1/4 milli-mole of oxygen)
M is the molarity of thiosulphate used as titrant
V is the volume of the sample bottle in cm^3, less $2cm^3$ to replace sample loss due to the addition of $2cm^3$ reagents
B is the volume of sample titrated in cm^3

To convert the concentration unit into $cm^3 \cdot dm^{-3}$ ($mg.l^{-1}$), the results obtained were multiplied by 32/1000 since 1 μM O_2= 32 μg $O_2 \cdot dm^{-3}$. Because some of the salinity and temperature data exceeded those in the range provided by Parsons et al (1984), a saturation value was calculated using the following equation (Weiss, 1970) :

$$\log_e C = A1 + A2(100/T) + A3\log_e(T/100) + A4(T/100) + S(B1) + B2(100/T) + B3(T^2/10000)$$

where: C = oxygen saturation value, as $mg.l^{-1}$
S = salinity, ppt
T = absolute temperature, i.e. °C+273.15
A1 - B4 are constants, i.e.

A1= -173.4292	B1 = -0.033096
A2= +249.6339	B2 = +0.014259
A3= +143.3483	B3 = - 0.0017
A4= - 21.8492	

Providing that both the dissolved oxygen concentration and the oxygen saturation are presented in the same units ($mg.l^{-1}$); the percentage saturation of oxygen may then be obtained by dividing the DO by the oxygen saturation value and multiplying by 100:

$$\text{Percentage saturation} = \frac{\text{Dissolved oxygen}}{\text{Oxygen saturation}} \times 100$$

2.2.2.4. Determination of chlorophylls and total carotenoids

The trichromatic spectrophotometric equations described here are based on the technique recommended by UNESCO (Lorenzen & Jeffrey, 1980) for near surface euphotic zone waters. However, this calculation is less useful for deeper water layers because of interference from detrital chlorophyll. The lower limit of detection using this method is <1% error (0.02 $mg.m^{-3}$) for chlorophyll-*a* and chlorophyll-*b*. In this equation, the chlorophyll-*c* represents total chlorophyll-*c*, i.e. *c*1 and *c*2 (Strickland and Parsons, 1968). The amount of carotenoids can only be approximated since these are a mixture of several compounds which may have different molar extinction coefficients (Parsons et al, 1984). Nonetheless, in this study the concentration of chlorophyll-a will be quoted mostly since it represents the majority of pigments carried over by phytoplankton.

As soon as possible on return to the laboratory, the polyethylene bottle containing the seawater sample was inverted into the Millipore filtering equipment containing a fibre glass filter (Whatman GF/C, as it was assumed initially that some phytoplankton would be difficult to extract), and filtered under 1/2 atmospheric pressure vacuum. Several (3-5) drops of dilute $MgCO_3$ solution were added to prevent acidity on the filter. Having been drained thoroughly by suction, the filter was placed in a 15-ml centrifuge tube, 10 ml of 90% acetone was added and the tube thoroughly shaken. It was then left to stand overnight in the dark and at a cool temperature (refrigerated at 4°C) for pigment extraction.

The following day, the contents of each tube were centrifuged at room temperature for 10 min. The clear supernatant was transferred into a 5-cm path length spectrophotometer cuvette and the extinction measured without

delay at room temperature to avoid misting of the optical cells. Measurements were made at the following wavelengths 750, 664, 647, 630, 510 and 480 nm. Following the standard procedure of Parsons et al (1984), each extinction was corrected for a small turbidity blank, by subtracting the 750 nm from the 664, 647 and 630 nm absorptions. The reading at 510 nm absorbance was similarly corrected by subtracting twice the reading at 750 nm absorbance and the reading at 480 nm was corrected by subtracted to three times the reading at 750 nm.

The amount of pigment in the original seawater samples was calculated using the following equations described by Parsons et al (1984) :

$$C_a = 11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630}$$
$$C_b = 21.03\ E_{647} - 5.43\ E_{664} - 2.66\ E_{630}$$
$$C_c = 24.52\ E_{630} - 1.67\ E_{664} - 7.60\ E_{647}$$
$$C_p = 7.60\ (E_{480} - 1.49\ E_{510})$$

$$\text{mg chlorophyll.m}^{-3} = \frac{Cxv}{Vx10}$$

where C_a, C_b, C_c and C_p are the amounts of chlorophyll-*a*, *b*, *c* and plant carotenoids in $\mu g.ml^{-1}$ ($\equiv mg.m^{-3}$, if a 1 cm light path cuvette is used) and E stands for the absorbances at different wavelengths in the spectrophotometer; *v* is the volume of acetone in ml, *V* is the volume of seawater in litres.

2.2.2.5. Particle size analyses

The method used, known as the pipette method, was modified from Piper (1950) by using converted wet sample weight instead of dry weight. Basically, the particles are graded into fractions by their settling velocities which are ultimately related to temperature. Morton and Morton (1983),

Boonruang and Janekarn (1983), McLusky (1989) and Marques et al (1993) graded the mean diameter of fine sand as 250-125μm, very fine sand 125-62.5μm, silt 31.2-3.9μm and clay 1.9-0.12μm. Here, sieving was carried out only to segregate the first fraction, i.e. fine sand. Subsequently silt and clay particles were obtained from the pipette samples with the very fine sand grains being the last fraction separated by a series of decantations. The analyses were conducted once a month in batches of 6 duplicated samples from three sites at a time. Whilst waiting for other samples to be collected, available samples were refrigerated in boxes to avoid water loss by evaporation. In order to limit within-core variability resulting from vertical gradients in sedimentary parameters, the analyses were carried out on well mixed sediment cores and distilled water was used throughout the analyses.

Basically a known weight of sediment was oven dried at 110°C for 12-16 hours and the percentage of moisture in the sample was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100$$

The percentage of moisture was therefore used as a correction factor for calculating 10 g dry samples required as a basic weight in the subsequent analysis. Wet samples (≡ 10 g of dry weight) were then weighed instead:

$$\text{Wet sample (g)} = \frac{\text{10 g dry weight}}{(100\% - \% \text{ Moisture})} \times 100$$

The sample was wet sieved on a 125 μm (≡50 BS mesh size) sieve and the fine sand which separated was then set aside to dry in an oven at 110°C for 12 hours and weighed as *oven dry fine sand.*

Individual particle separation is a pre-requisite for effective particle size analysis. During preliminary tests, a number of dispersant agents such as

hydrogen peroxide, ammonia and sodium hydroxide (Piper, 1950) had been previously tried and these indicated that sodium polyphosphate $(NaPO_3)_6$ produced the most satisfactory particle dispersion. Using $(NaPO_3)_6$ the suspension dispersed completely and appeared smooth under microscopic examination. During analyses, a mercury thermometer of 0.1°C accuracy dipped in the water column in a tall beaker next to the sedimentation cylinders was used to assess the suspension temperature.

All the filtrate containing fine suspended material was funneled into a Pyrex sedimentation cylinder (Appendix 2) and 10 ml of 10% $(NaPO_3)_6$ was added (Piper, 1950), the suspension diluted to 1000 ml and left overnight to allow the reaction to proceed. The following day, the suspension was re-stirred by inverting the cylinder in steady but vigorous up and down strokes until all sediment was removed from the bottom of the cylinder, usually 45 sec - 1 min. The time of commencement of silt and clay sedimentation for the first pipette sample was noted from the corresponding water temperature recorded (Table 1 in Appendix 2). Just before sampling, the tip of a 39 cm-stemmed pipette was lowered to 28 cm below the surface of the suspension and the pipette filled by applying gentle suction to it. The 25ml sample collected was delivered into a pre-weighed aluminium dish, evaporated to dryness on a water bath, transfered to an oven at 110°C, dried overnight, cooled in a dessicator and weighed. The increase in weight was recorded as *oven-dry silt and clay.* To obtain the amount of silt and clay in 1 litre slurry (≡10 g dry sample), this weight was multiplied by 40, assuming the density of the suspension is 1.

The second pipette sample was always withdrawn after an overnight period of sedimentation. Sampling time was determined from Table 1

(Appendix 2) which is based on the average air temperature during sedimentation. The same procedure was applied as for the first pipette sample, and this second pipette sample weighed as *oven-dry clay*. The amount of clay in 1 litre slurry (≡10 g of dry sample) is:

$$\text{Clay. } l^{-1} = [(\text{oven dry clay}) \times 40] - 1.0526$$

where 1.0526 is a correction factor for the weight of sodium polyphosphate used in dispersing the suspension. This should be deducted from the clay fraction since most of the dispersant remains in solution. The amount of oven dry silt in 1 litre slurry then corresponds to:

$$\text{Silt. } l^{-1} = [(\text{oven dry silt and clay}) - (\text{oven dry clay})] \times 40$$

After withdrawing the second pipette sample from the sedimentation cylinder, the suspension was removed to within 3-4 cm of the bottom, by means of a siphon tube connected to a filter pump. The lower end of the siphon tube should be bent upwards to prevent disturbing the sediment on the bottom of the cylinder. To the cylinder was also added any of the very fine sand which had been separated from the fine sand by re-sieving the oven dry material. The cylinder was then re-filled with water to the 10 cm mark, stirred, and after 10 min was decanted. The corresponding time of decantation for the very fine sand was obtained from the second column in Table 1 (Appendix 2) after determining the water temperature. This operation was repeated until the supernatant became transparent and usually only a negligible amount of material remained in suspension. The last decantations were timed properly, so that the correct time interval occurred in the middle of the process. The very fine sand obtained was evaporated to dryness on a water bath, dried overnight in an oven at 110°C, cooled in a dessicator and weighed as *oven-dry very fine sand.*

After determining each fraction in the samples i.e. fine sand, very fine sand, silt and clay; the actual weight of the fractions to the original weight of moisture free basis of sample (≡10 g), was tabulated. Both the values of oven dry fine sand and very fine sand are used without correction, since the first fraction was separated before the addition of dispersant agent and values of the very fine sand were measured after such a series of decantation that removed any trace of dispersant. The summation of these four fractions should approximate to 10. If the total is less than 9.85 or more than 10.2, the analysis should be regarded with suspicion and re-checked. The replicates should agree to within ≤0.1 gram (Piper, 1950).

Since the physico-chemical variables such as temperature, salinity, rainfall, pH and oxygen saturation values control the distribution and abundance of estuarine organisms directly as well as through their influence on food availability (Stanley, 1970; McLusky, 1989), the relationships between such factors and the concentration of chlorophyll-a in the water were assessed applying the nonparametric Spearman product moment correlation for time series data. A combination of QuattroPro v.4.0, Harvard Graphic v.3 and Minitab Release 8.21 software packages (Ryan et al, 1985) were employed for data handling, calculating numerous descriptive statistics, and assessing the significance and direction of differences in derived parameters between sites.

2.3. Results

2.3.1. Population density and pattern of dispersion

The population density of *A. granosa* in Tapak is very low. It was only possible to collect some specimens randomly yet their numbers were

frequently not adequate for a complete monthly sample analysis. Nevertheless, this locality was retained as one of the study sites since other localities are not accessible from Semarang. Furthermore, due to its critical position in the city plan development of Semarang as the capital of Central Java Province, any ecological study in this particular site would serve as a reference in the future.

In Wedung, clam density varied from zero (February 1993) to a maximum of 45 indiv.m^{-2}, with an average density of less than 10 individuals.m^{-2} (i.e. 9.19 indiv.m^{-2})· Highest densities were found in August, i.e. 11.5 (in 1991), 29.64 (in 1992) and 30.85 (in 1993) indiv.m^{-2}. The densities immediately after peaks in August 1992 and November 1992 were remarkably low (Fig. 5A) due to commercial harvesting. However, low densities during December 1992 and February 1993, i.e. 0.18 and 0.0 indiv.m^{-2} were more likely to be caused by low salinities due to the heavy rainfall. The population densities of *A. antiquata* in Bandengan were relatively constant compared to those of *A. granosa* in Wedung. The density in Bandengan ranged from 0-10 indiv.m^{-2} with an average of 2.46 indiv.m^{-2} and a peak of 3.5 indiv.m^{-2} in May 1992 (Fig. 5B).

Figure 5C shows that the distribution of *A. antiquata* in Bandengan was confined mainly to the intertidal areas. No attempt was made to quantify the horizontal distribution of *A. granosa* at Wedung as it was for *A. antiquata* in Bandengan. The relative values obtained from several observations in the field suggest that in Wedung the distribution of *A. granosa* extends 25m beyond low water mark into the subtidal areas of ca. 4m depth. At Tapak, however, the blood clam beds are found in the intertidal zone but are restricted to an area of about 900m^2 of the total enclosed flats (ca. 2 km^2)

behind the main sandbar. Their absence subtidally is perhaps because the bottom sediment seaward off the sandbar is composed mainly of coarser sediment. Moreover, the pronounced heterogeneity of this site is caused by the discharge of sediment loads from the dredging and construction work for an international harbour Tanjung Emas and marina in Semarang, some 2 km north-east of the site. Sediment bars are formed temporarily, changing the pattern of currents and leading to accumulation of coarse sand subtidally as well as intertidally on the potential beds behind the Tirang Cawang Island (Fig. 3). Subtidally, the environmental conditions may become even less favourable due to the high levels of pollutants discharged by the industrial areas upstream, about 3 km southward of the site.

In Bandengan, *A. antiquata* is dispersed horizontally along the beach in the mid intertidal areas between mean high water neap and mean low water of neap tide. *A. antiquata* does not occur subtidally nor in the high intertidal areas (Fig. 5C). In comparison to Wedung, the density of *A. antiquata* in Bandengan remained relatively constant during the study period, albeit at a much lower density (compare Figs. 5A & B).

Table 1 illustrates the indices of dispersion for *A. granosa* and *A. antiquata* in Wedung and Bandengan throughout the study period. The significant values of $Id > 1$ shown by *A. granosa* populations on November 1991, September 1992 and July 1993 suggest a patchy dispersion. Those months coincide with the peak spawning season for this particular species during the study. The sudden decrease in the index for example from November 1991 to March 1992, September 1992 to November 1992 and July 1993 to August 1993, reflects the thinning in the density of individual blood clams due to commercial harvest. Throughout the rest of the year

Table 1: Indices of dispersion ($Id = s^2/\tilde{N}$) for *A. granosa* from Wedung population and *A. antiquata* from Bandengan (<1 discrete, 1 random and >1 patchy)

a. *A. granosa*

Date	n	*I*d
26 August 1991	14	0.938
3 November 1991	14	2.964*
15 March 1992	14	1.000
14 June 1992	14	0.643
23 August 1992	14	0.921
27 September 1992	14	2.531*
20 November 1992	14	0.857
20 December 1992	14	0.929
14 February 1993	10	0.392
11 July 1993	13	2.492*
8 August 1993	13	1.147

* significant at P0.05, df13= 2.16
df 9= 2.262
df12= 2.179

b. *A. antiquata*

Date	n	*I*d
12 September 1991	20	1.243
12 January 1992	20	2.458*
1 June 1992	20	2.226*
1 November 1992	15	1.046
3 January 1993	19	1.154
4 July 1993	19	1.681

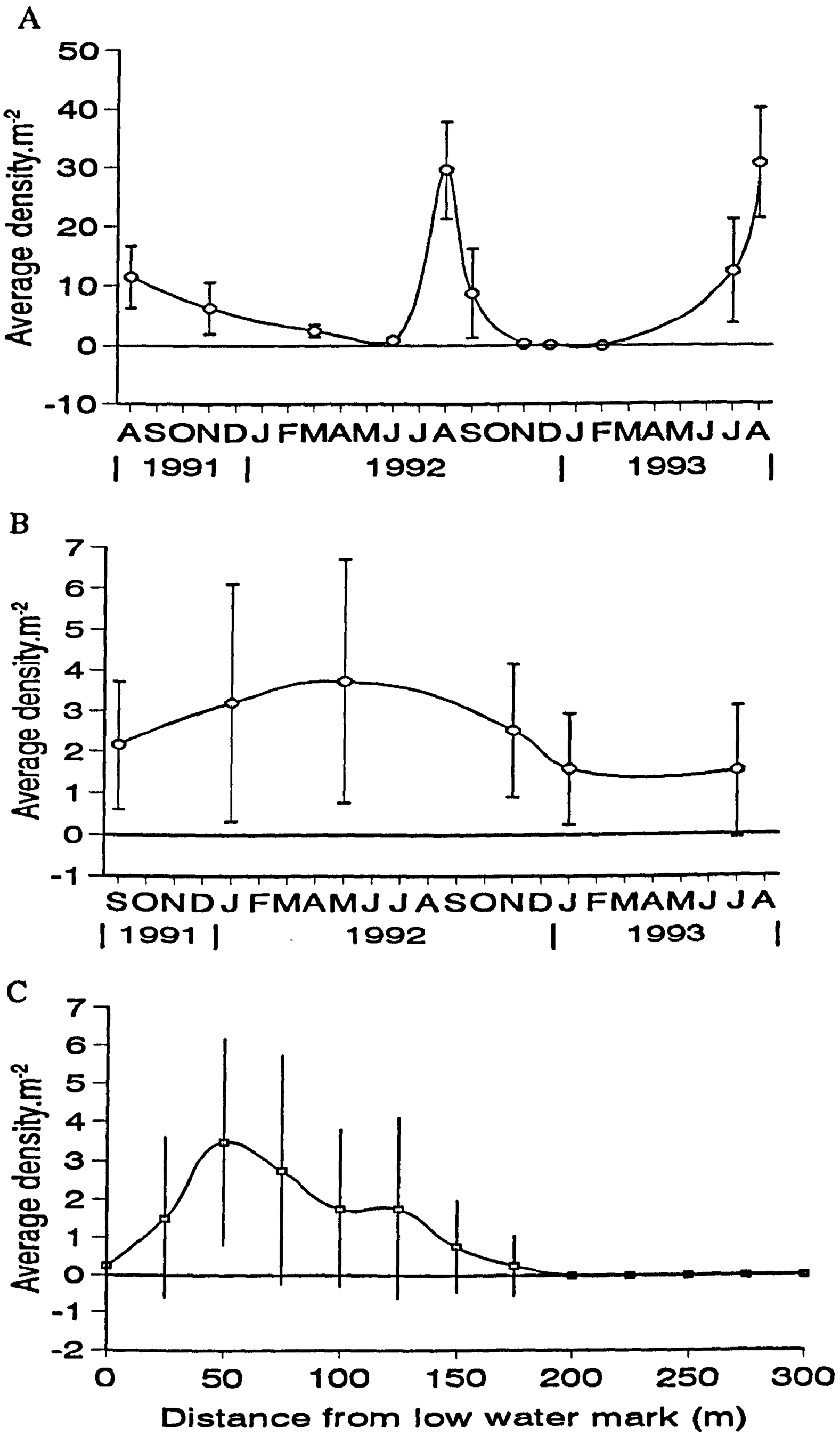
* significant at P0.05, df19= 2.093
df14= 2.145
df18= 2.101
n = number of observations (quadrat of $1m^2$)

Figure 5: Density and spatial distribution of *A. granosa* and *A. antiquata.*

A. Seasonal variation in estimates of the average density of *A. granosa* from the Wedung population. ± Standard Deviation

B. Seasonal variation in estimates of the average density of *A. antiquata* from the Bandengan population. ± Standard Deviation

C. The horizontal distribution of *A. antiquata* at Bandengan plotted against distance from low water mark of spring tide, showing the highest density of individuals occurred at about 50 m from low water.


A
Average density.m-2
50
40
30
20
10
0
-10
ASONDJFMAMJJASONDJFMAMJJA
1991
1992
1993
B
Average density.m-2
7
6
5
4
3
2
1
0
-1
SONDJFMAMJJASONDJFMAMJJA
1991
1992
1993
C
Average density.m-2
7
6
5
4
3
2
1
0
-1
-2
0
50
100
150
200
250
300
Distance from low water mark (m)

clams were evenly distributed within the small areas of study. In March 1992 and August 1993 the index of dispersion approached randomness (Id= 1.00 and 1.147 respectively). These values, however, cannot be taken as proof of a random pattern, since the absence of significant departures may have resulted from the limitations of the sampling methods used. The populations of *A. antiquata* in Bandengan showed a relatively contagious distribution (Id >1) although it was only significant in certain months of the year, e.g. January and June 1992, Table 1.

2.3.2. Associated macrofauna in the clam beds

The composition of the macrofaunal community associated with two natural populations of *A. granosa* in Wedung and Tapak, and *A. antiquata* in Bandengan are presented qualitatively in Tables 2, 3 and 4. The relative abundance of the organisms is based on the average total area of each sampling plot surveyed for both *Anadara* during the two year study, i.e. 150m^2. This was so because they were collected as part of the routine sampling programme, which did not involve the use of a quadrat.

The sampling technique used excluded the collection of animals measuring less than 10 mm in their smallest dimension. Density estimates for fish, penaeid shrimps and brachyuran crabs, all of which are inefficiently sampled using the techniques employed here, were assessed from the number caught by chance on each sampling occasion. These estimates were then adjusted to the number captured by the local fishermen using hand nets of 5mm mesh size which equated to a fishing effort of ca. 12m^2·unit effort^{-1}.

There are some similarities regarding the faunal composition of the community, particularly for bivalve species in Wedung and Tapak, and crab species in the three sites. Only samples of *A. granosa* and *A. antiquata* were

estimated quantitatively (see Fig. 5). However, in Tables 2-4 both species are presented qualitatively in order to compare them to other macrofaunal members of the community.

The common species collected were *A. granosa* and *Scapharca cornea* in Wedung and Tapak, and *A. antiquata* and *Barbatia barbata* in lower numbers in Bandengan. Apart from the two species studied, eight other species of the family Arcidae were found in the study sites (denoted with a symbol • in Tables 2-4). Four of these species co-occur at Wedung. Tapak has two, one of these, *Scapharca cornea*, was also found in Wedung. This species lives subtidally and was probably washed inshore into the shallow subtidal where the *A. granosa* occurred. Three other species of anadarinids were found in Bandengan.

The arcids found in Tapak and Wedung were quite similar with regard to their moderately heavy, subovate-globular shells, shell ornamentation and also in their relative shell thickness. None of them displayed a byssal system. Instead, they were slightly inequivalve apart from *A. granosa*. The species of anadarinids which appeared to prosper in Bandengan were those species characterised by either having thick and heavy shells like *Arca ventricosa* and *A. antiquata* or those of smaller size, having a laterally compressed mytilid form and strong byssus stem such as *Barbatia barbata* and *B. fusca*. Their byssus fibres are fused into a tough membrane that quite often attaches the shell upright with the ventral margin against the rock. In this study, the only species of anadarinids which occurred intertidally and close to the head of the estuary in Tapak where fresh water enters (*Anadara sp.* 2, Table 3) was smaller and had a trapezoid outline. The periostracum of its equivalved shell is dark brown. The shell is relatively thin with cross bar shell sculpture.

Two ecomorphs of *A. granosa* were found in this study. Both were collected regularly from Wedung and Tapak. Apart from their outline they look similar to each other, though one is more elongate than the other. In Wedung, the rounded form was mainly collected from the marginally subtidal areas whereas the elongated clams were mostly subtidal. However, in Tapak these two morphs appeared to co-occur with the elongated form extending its distribution into the shallow subtidal areas. The periostracum of the round form is brownish yellow and the inner part chalky white. The elongated form has a sub-elliptical outline, an off-white outer colour whilst the inner shell where the mantle is attached is yellowish, particularly when the animals are reproductively active. Further discussions regarding their allometry is presented in Chapter IV.

Since these tables list only animals collected as part of the normal monthly sampling programme, other animals which are not sampled by the techniques used may also be present. These may include ephemeral members of the community, such as the water snake (*Enhydris sp.*), wading birds like *Tringa nebularis*, *Egretta gazetta* and *Charadrius sp.* in Wedung and Tapak, and rays in Bandengan which are regularly observed at the sites. On one occasion, some catfish of the genera *Plotosus* (family Plotosidae) and *Epinephelus* (family Serranidae) caught in Tapak were found to contain a number of *A. granosa* shells within their stomachs. In Wedung, a large numbers of gastropods *Babylonia areolata, Natica tigrina* and *N. maculosa* were continually landed by the fishermen from offshore subtidal areas. The latter has been reported as a potential predator of *A. granosa* in Malaysia (Broom, 1981, 1982 a, 1983a,c).

Table 2: Composition of the macrofaunal community associated with a natural population of *A. granosa* in Wedung

Phylum	Classes	Genus/Species	Density
Mollusca	Gastropoda	*Cerithidea cingulata* (Gmelin 1790)	++
		Nassarius dorsatus (Roeding)	++
		Nassarius pullus (Linnaeus)	+++
		Melongena pugilina Born	++
		Architectonica sp.	+
		Bedeva blosvillei	+
	Bivalvia	*Anadara granosa* (Linnaeus)	+++
		Clausinella chlorotica (Philippi, 1849)	+++
		Chamelea gallina (Linnaeus, 1758)	++
		Tapes literatus (Linnaeus, 1758)	++
		Meretrix lamarckii Deshayes	++
		Tapes philippinarum (Adams & Reeve, 1850)	++
		Dosinia japonica (Reeve, 1850)	++
		Paphia undulata (Born, 1778)	++
		Trachycardium asiaticum (Bruguiere1843)	++
		Marcia flammea (Gmelin, 1791)	++
		Placuna placenta (Linnaeus, 1758)	++++
		Atrina seminuda (Lamarck, 1819)	+
		Pteria tortirostris (Dunker, 1848)	+
		Unidentified Anomiid	+
		Saccostrea cucullata Born	+
		• *Scapharca cornea*	++
		• *Cunearca pilula*	++
		• *Anadara* cf. *ehrenbergi* (Dunker, 1868)	+
		• *Anadara sp.* 1	++
Arthropoda	Chelicerata	*Tachypleus gigas* Muller	++
	Decapoda	*Thalamita sp.*	++
		Scylla serrata	++
		Portumus pelagicus	+++
		Uca sp.	++++
		Palaemonetes sp.	++++
		Penaeus sp.	+++
Echinodermata	Asteroidea	*Asterias sp.*	+
Brachiopoda		*Lingula unguis* (Linnaeus)	++
Pisces		*Boleophthalmus boddarti* (Pallas)	++++

Qualitative density estimated per $150m^2$:

++++ abundant, ≥ 50 individuals, ++ fair, 1-5 individuals,

+++ common, 6-10 individuals, + rare, ≤ 1 individual.

(•) other species of anadarinid apart from *A. granosa* and *A. antiquata*

Table 3: Composition of the macrofaunal community associated with a natural population of *A. granosa* in Tapak

Phylum	Classes	Genus/Species	Density
Mollusca	Gastropoda	*Telescopium telescopium* (Linnaeus)	+++
		Turritella terebra Linnaeus	+
		Cerithidea cingulata (Gmelin 1790)	++++
		Nassarius pullus (Linnaeus)	++
		Melongena pugilina Born	++
		Terebralia sulcata	++
	Bivalvia	*Anadara granosa* (Linnaeus)	++
		Clausinella chlorotica (Philippi, 1849)	+++
		Chamelea gallina (Linnaeus, 1758)	++
		Tapes literatus (Linnaeus, 1758)	+
		Tapes cf *platyptycha* (Pilsbry)	+
		Meretrix lamarckii Deshayes	+
		Tapes philippinarum (Adams & Reeve 1850)	+
		Paphia undulata (Born, 1778)	+
		Sinovacula rugosa (Linnaeus, 1767)	+
		Perna viridis (Linnaeus, 1758)	+
		Modiolus philippinarum (Hanley, 1843)	+
		Placuna placenta (Linnaeus, 1758)	+
		Unidentified Anomiid	++
		Saccostrea cucullata Born	+++
		Crassostrea gigas (Thurnberg, 1793)	+++
		• *Scapharca cornea*	+
		• *Anadara sp.* 2	++
		Malleus albus Lamarck, 1819	++
Arthropoda	Chelicerata	*Tachypleus gigas* Muller	+
	Decapoda	*Palaemonetes sp.*	+++
		Penaeus sp.	+++
		Thalamita sp.	++
		Scylla serrata	+++
		Portumus pelagicus	++
		Uca arcuata	+++
		Ocypode sp	+++
Brachiopoda		*Lingula unguis* (Linnaeus)	++
Pisces		*Boleophthalmus boddarti* (Pallas)	+++
		Plotosus canius H.B.	+
		Epinephelus lanceolatus (Bloch)	+
		Mugil cephalus	++
		Chanos chanos	++
		Periophthalmus cantonensis	++

For abundance key see Table 3. (•) other species of anadarinid apart from *A. granosa* and *A. antiquata.*

Table 4: Composition of the macrofaunal community associated with a natural population of *A. antiquata* in Bandengan

Phylum	Classes	Genus/Species	Density
Mollusca	Gastropoda	*Cerithidea coralium* (Kiener)	+++
		Littorina sp.	+++
		Natica maculosa (Linnaeus)	+
		Cypraea cf. *arabica*	+
		Turbo cinerea	+++
		Monodonta australis	+++
		Tectus pyramid	++
		Clypeomorus humilis	++
		Nerita undata	++
		Trochus sp.	+++
		Hemifusus ternatana	++
		Morula musiva	++
		Batillaria cumingii	++
		Umbonium vestiarium (L.)	++
	Bivalvia	*Anadara antiquata* L.	+++
		Gafrarium tumidum Roding, 1798	+++
		Periglypta crispata (Deshayes, 1854)	+
		Ruditapes variegatus (Sowerby, 1852)	+
		Trachycardium flavum (Linnaeus, 1758)	+
		Trachycardium enode (Sowerby, 1834)	+
		• *Barbatia barbata* (Linnaeus, 1758)	+
		• *Arca ventricosa* Lamarck, 1819	+
		• *Barbatia fusca* (Bruguière, 1789)	++
		Spondylus cf. *aurantius* Lamarck, 1819	+
		Mactra sp.	+
Echinodermata	Holothuroidea	*Holothuria leucospilota*	++++
Arthropoda	Decapoda	*Thalamita sp.*	+++
		Calappa sp.	+
		Portunus pelagicus	+++
		Pinnotheres sp.	+++
		Callinectes sp.	++

For abundance key, see Table 3.

(•) other species of anadarinid apart from *A. granosa* and *A. antiquata.*

It can be seen from Tables 2-4 that both *A. granosa* in Wedung and *A. antiquata* in Bandengan were invariably the numerically dominant species and more likely they have the greatest biomass of all species, although they will be affected by year-to-year fishing activities. The *A. granosa* population in Tapak however, can be considered as an impoverished community in which the clam population occurs at low density and is dominated by large female specimens (see Chapter III, Fig. 14B). Yet in terms of species numbers, it can be seen that Tapak has the higher faunal richness than the other two sites although the density of individual species is low. Bandengan has the largest variety of gastropods amongst the three sites. However, none of the species found at Bandengan were seen at either Wedung or Tapak (Tables 2-4). At Bandengan, the common observed 'conveyor-belt' species *Holothuria leucospilota* may serve as a sediment re-worker in this sandy beach. The mytilids were rarely found, and when present were confined to single individuals of two species found in Tapak, *Perna viridis* and *Modiolus philippinarum*. Wedung seemed to be a favourable place for *Placuna placenta*, which is collected commercially both for their meat and shells. Meanwhile, the ten species of venerids noted in the tables were commonly observed at all three sites. However, the species which occurred in Bandengan were those having thick and heavy shells, like *Gafrarium tumidum*, *Periglypta crispata* and *Spondylus sp.* (Pectinoidea). These were found on the landward part of the middle shore where coarse sand was admixed with silt.

The presence of the barnacle *Balanus amphitrite* upon both the rounded and elongated forms of *A. granosa* was negligible (4 out of 7450 specimens) and it was never found on *A. antiquata*. The population of *A. antiquata* in

Bandengan, however, was found to be infested by an unidentified pinnotherid crab. This pea crab was never found in *A. granosa* from Tapak and was observed only once in an isolated Wedung specimen. Further discussions regarding this symbiont are presented in Chapter III.

2.3.3. Environmental characteristics

The tidal regime at the study sites is a mixed semi-diurnal one with spring tides occurring twice each month (Fig. 6). The tidal amplitude between high and low water of spring tides is less than a metre (ca. 80cm; see Fig. 6) whilst the range during the intervening fortnightly neap tides is much less. According to the tide tables, successive high waters during spring tides may be separated by between 8 and 24 hours. Sampling in Wedung, Tapak and Bandengan was carried out in the shallow subtidal areas during low tide.

A summary of the physico-chemical parameters measured for Wedung, Tapak and Bandengan during the period of this study, August 1991-August 1993 is given in Table 5. Sediment temperature was normally slightly lower than the surface sea water temperature; although occasionally they were similar. Throughout the study period, the range of sea temperature in Wedung was slightly lower (27-36°C) than in Tapak and Bandengan, i.e. 28-40°C with an overall average of 31.9°C, 32.9°C and 32.3°C respectively (Table 5).

In Wedung, Tapak and Bandengan, the rainy season extends approximately from November, which is the onset of the north-east monsoon, to April and the dry season from May to October in Wedung and Bandengan, whereas in Tapak, perhaps due to the hilly nature of the coastline, the dry season was shorter than at the two other sites (Figs. 7F-9F; Plate 2B; BPTP-V, 1993). During the rainy season in 1992, January and

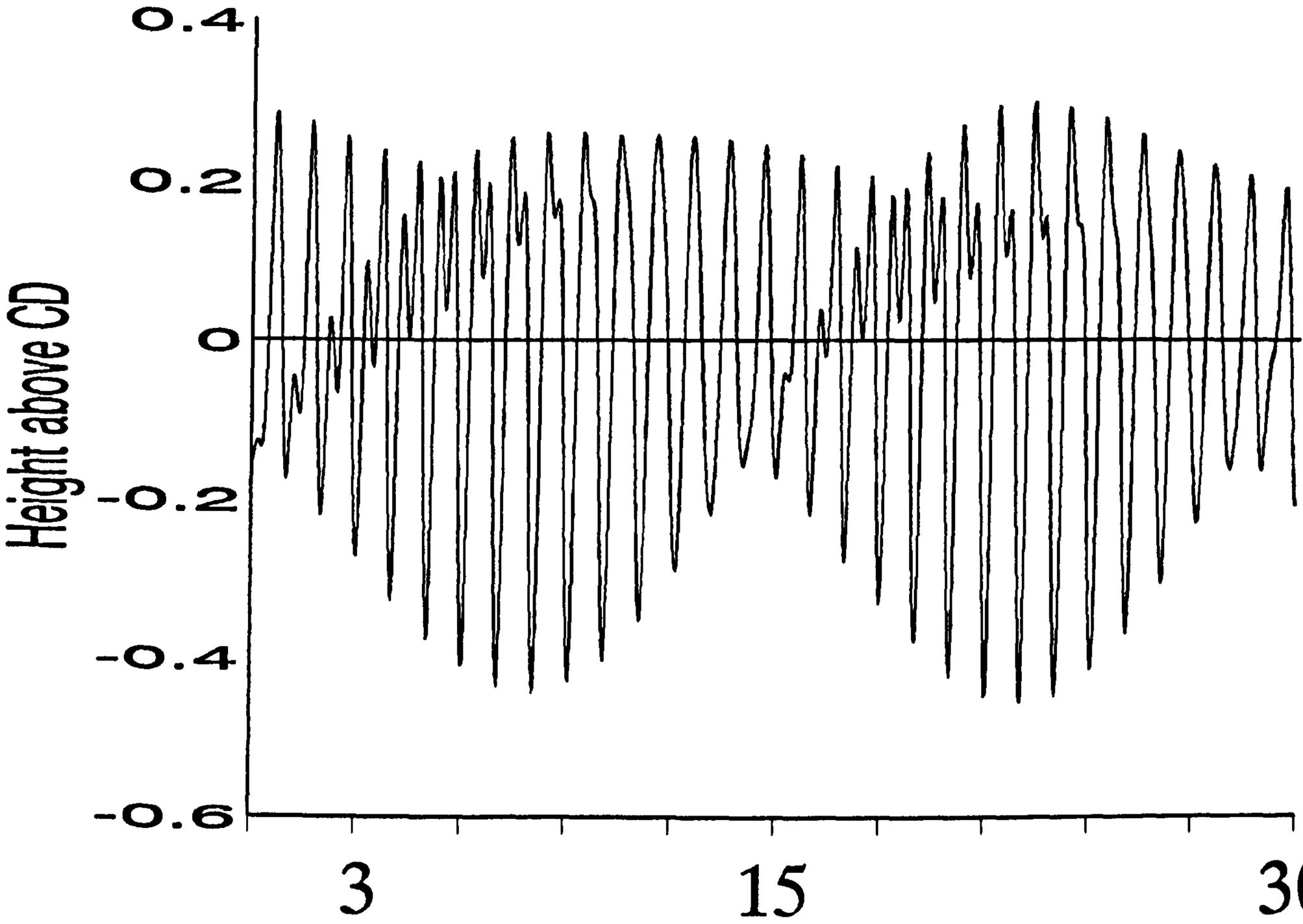


Figure 6: A typical tidal model for Semarang waters; the amplitude of the spring and neap tidal component of the mixed semidiurnal tide shows a prevailing diurnal pattern.

Table 5: Overall summary of the physico-chemical parameters at the study sites, between August 1991-August 1993. Each figure is the biennial mean and the standard deviation of 24 readings.

Parameters	Unit	Wedung	Tapak	Bandengan
Sea Water Temp.	°C	31.86 ± 2.39	32.87 ± 3.12	32.27 ± 3.52
Sediment Temp.	°C	30.90 ± 2.50	31.91 ± 2.96	31.44 ± 3.39
Sea Water Salinity	‰	26.76 ± 9.80	28.68 ± 5.37	31.17 ± 3.97
pH	-	8.18 ± 0.40	8.24 ± 0.39	8.29 ± 0.29
Dissolved Oxygen	$cm^3.dm^{-3}$	2.90 ± 0.54	2.48 ± 0.52	2.66 ± 0.56
Oxygen Solubilities	$cm.^3dm^{-3}$	4.42 ± 0.17	4.29 ± 0.19	4.30 ± 0.26
Oxygen Saturation	%	65.47 ±11.47	57.25 ± 11.97	62.26 ± 13.48
Chlorophyll-a	$mg.mm^{-3}$	1.85 ± 1.16	1.68 ± 0.92	0.26 ± 0.20
Monthly Rainfall	$mm^3.cm^{-2}$	150.10 ± 142.50	219.80 ± 156.60	215.30 ± 257.80
Fine Sand	gram%	1.50 ± 2.70	13.18 ± 0.25	68.32 ± 8.76
Very Fine Sand	gram%	19.74 ± 10.94	19.76 ± 7.97	19.59 ± 6.76
Silt	gram%	28.80 ± 6.79	21.60 ± 8.63	4.35 ± 3.91
Clay	gram%	51.23 ± 10.25	46.35 ±14.85	8.54 ± 4.24
Water content in the sediment	%	47.91 ± 6.73	50.99 ± 6.89	24.75 ± 1.57

February were the coldest months when the sea water temperature suddenly dropped although this change was less conspicuous in 1993 (Fig. 7A-9A). The highest recorded surface water temperature in Tapak was in the rainy season (November-December 1991 and March 1992), the same as in Bandengan (December 1991, March and April 1992), whereas in Wedung it occurred regularly as a yearly phenomenon during the rainy season (December 1991 and November 1992).

The salinity profile from Wedung shows the characteristics of an estuary receiving large quantities of fresh water run-off in contrast to the relatively less fluctuating one in Bandengan, a sandy beach having only a few small streams discharging into it (Fig. 4, compare Figs.7B-9B). In the middle of the rainy season in Wedung there was a noticeable and sudden fall in salinity (December 1991 and 1992). In December 1992-January 1993 prolonged and very heavy rainfall caused severe flooding in all the study sites, whilst in February 1993 local rain continued for several days and subsequently resulted in another flood in Wedung. Meanwhile, during the dry season (May-September) the salinity was relatively constant with some fluctuation due to sporadic rainfall and the discharge of several big rivers into Wedung Bay. In comparison to the salinity during the dry season in Wedung, salinity fluctuations in Tapak and Bandengan were less varied, perhaps because of the lack of fresh water entering these areas from adjacent rivers, coupled by relatively little rainfall during the dry season in Bandengan (1.0-60.5 $mm^3 \cdot cm^{-2}$, BPTP-V, 1993). It was only after such a prolonged heavy rainy season that caused floods at the end of December 1992 - January 1993, when the salinity dropped in both Tapak and Bandengan (Figs. 7, 9).

Figure 7: Seasonal variability of some physical parameters in Wedung during the study period August 1991 to August 1993.

A. Surface sea water (■) and sediment temperature (☒)

B. The profile of surface sea water salinity (●)

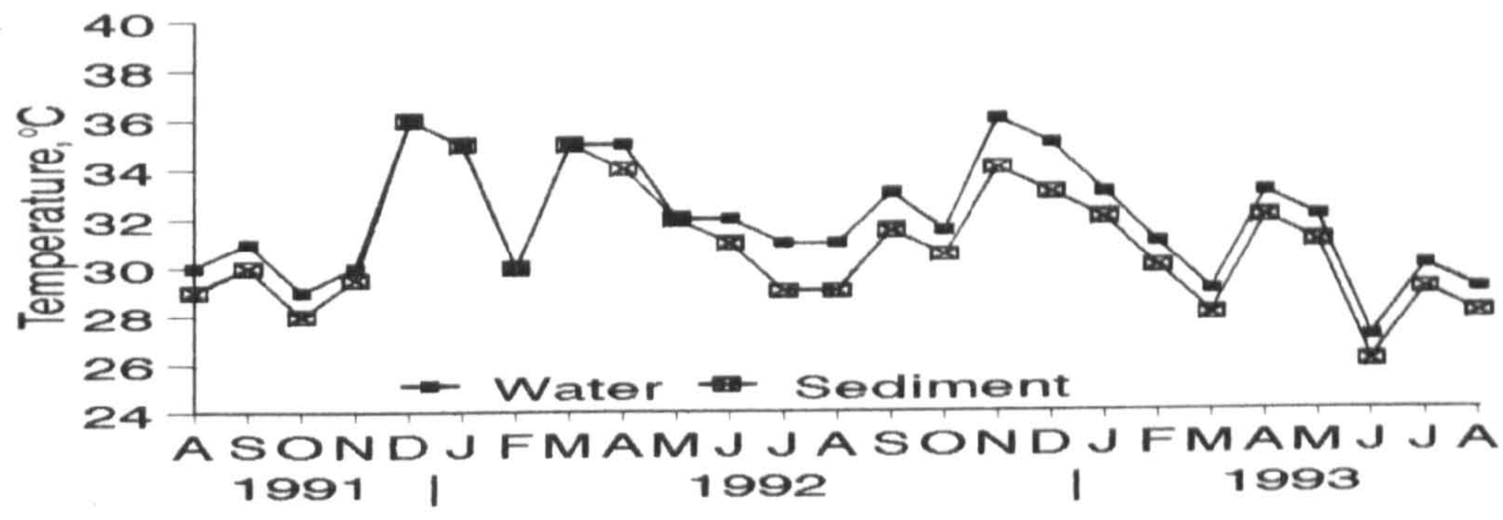
C. The pH (☒) of surface sea water

D. The seasonal variation of percentage oxygen saturation (●)

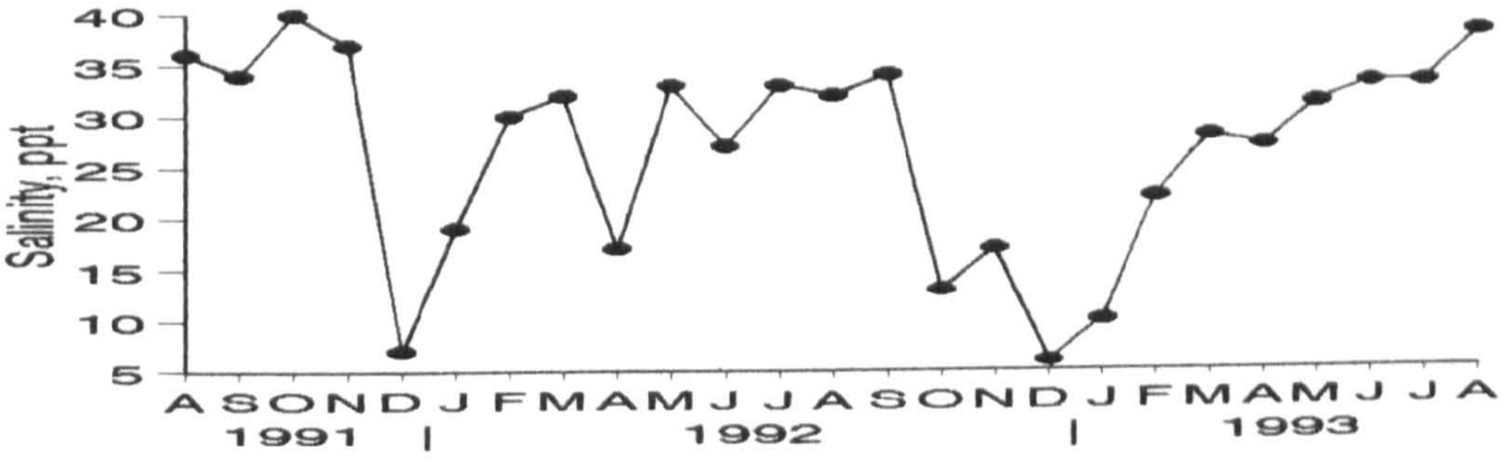
E. The seasonal variation of surface sea water concentration of chlorophyll-*a*. Each point is the mean ± Standard Error of three readings.

F. The total monthly rainfall in Demak, the nearest town to Wedung

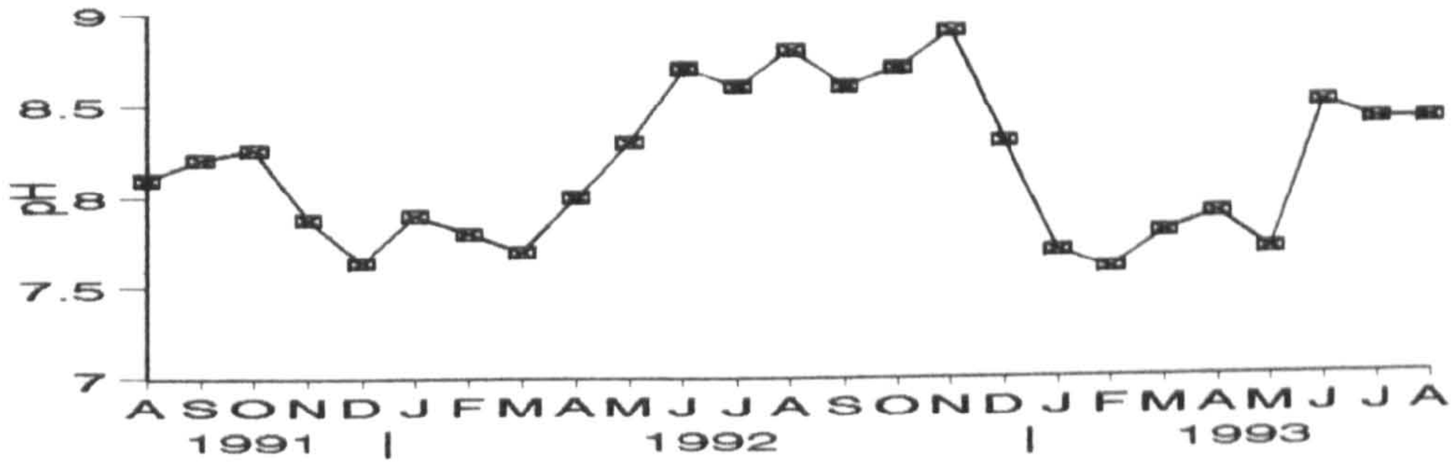
A


Temperature, °C
40
38
36
34
32
30
28
26
24
Water
Sediment
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

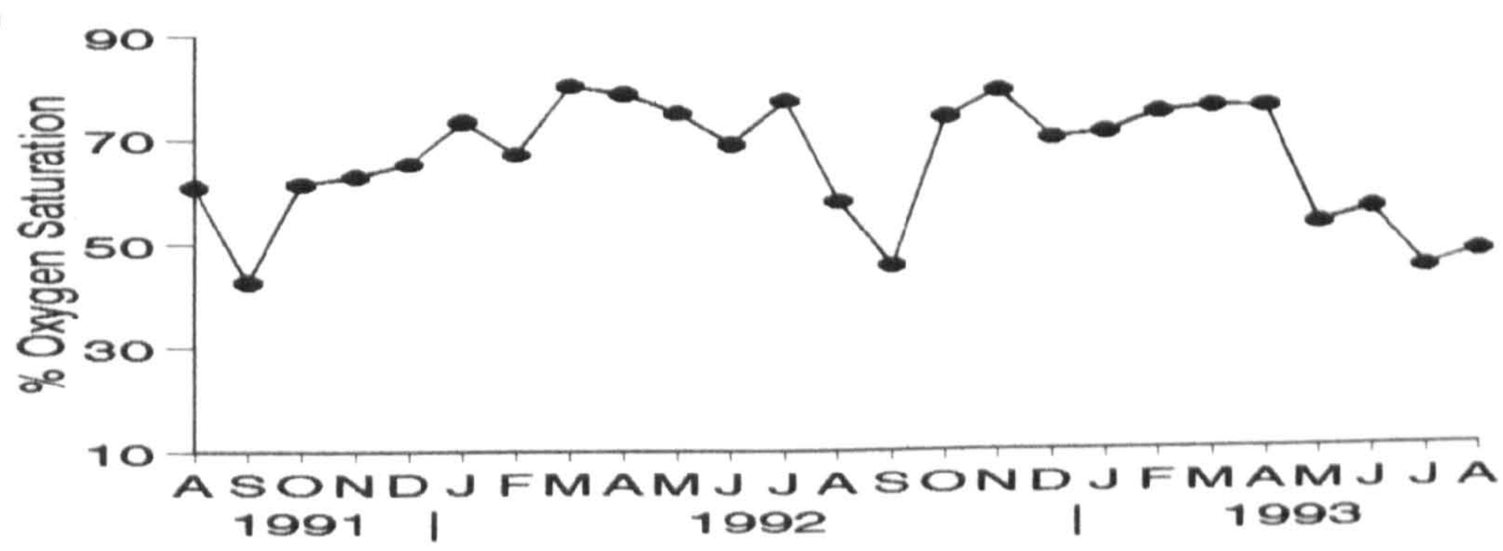
B


Salinity, ppt
40
35
30
25
20
15
10
5
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

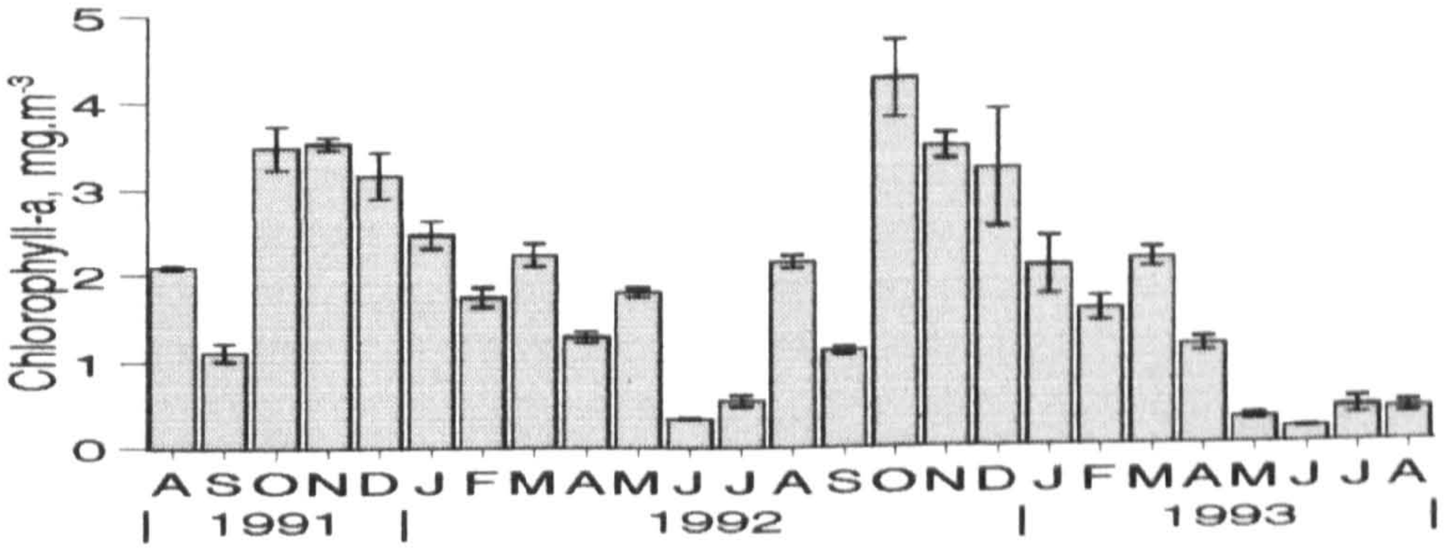
C


pH
9
8.5
8
7.5
7
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

D


% Oxygen Saturation
90
70
50
30
10
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

E


Chlorophyll-a, mg.m-3
5
4
3
2
1
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

F

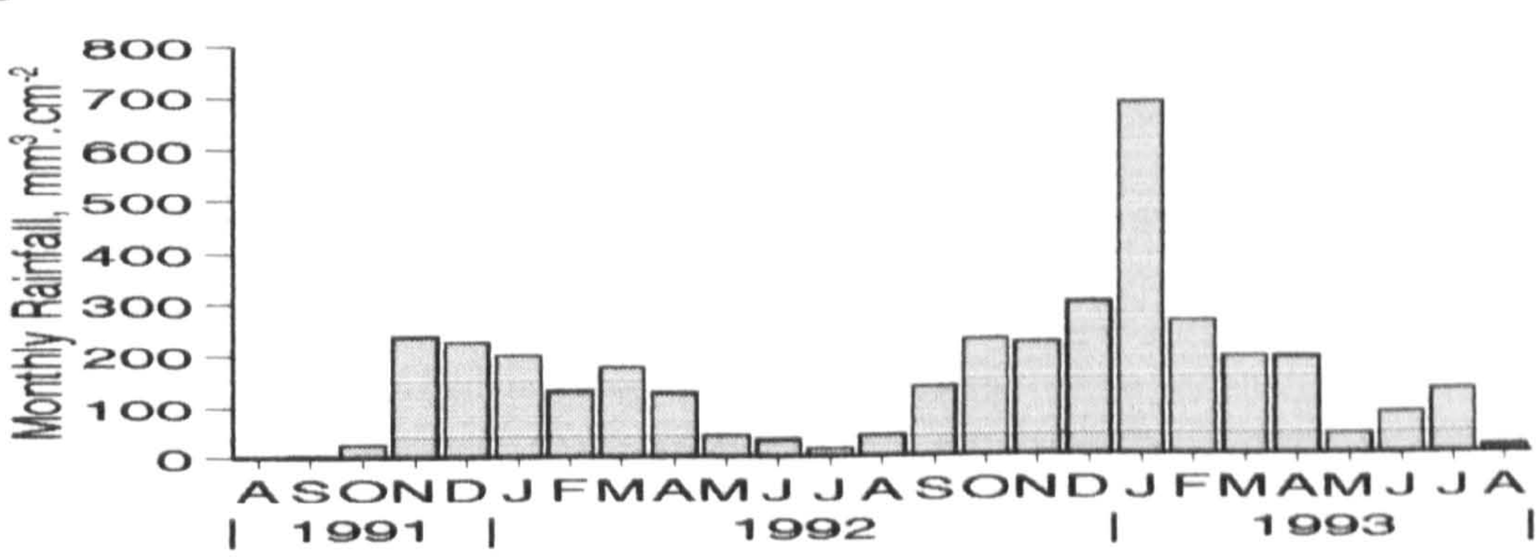

Monthly Rainfall, mm3.cm-2
800
700
600
500
400
300
200
100
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

Figure 8: Seasonal variability of some physical parameters in Tapak during the study period August 1991 to August 1993.

A. Surface sea water (■) and sediment temperature (☒)

B. The profile of surface sea water salinity (●)

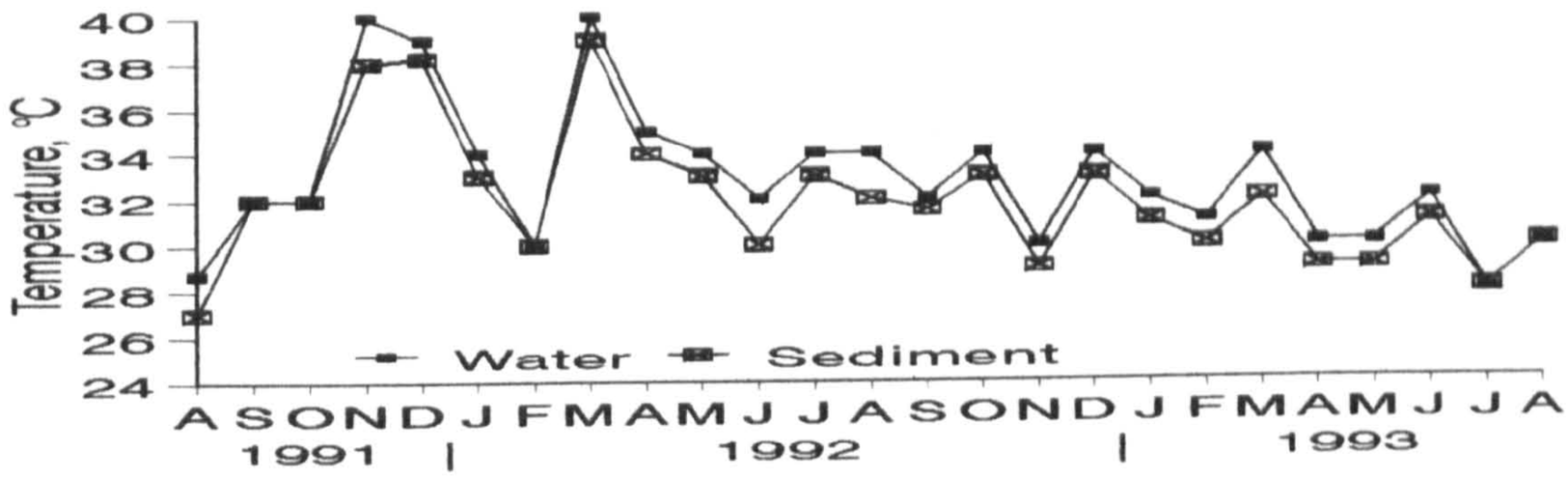
C. The pH (☒) of surface sea water

D. The seasonal variation of percentage oxygen saturation (●)

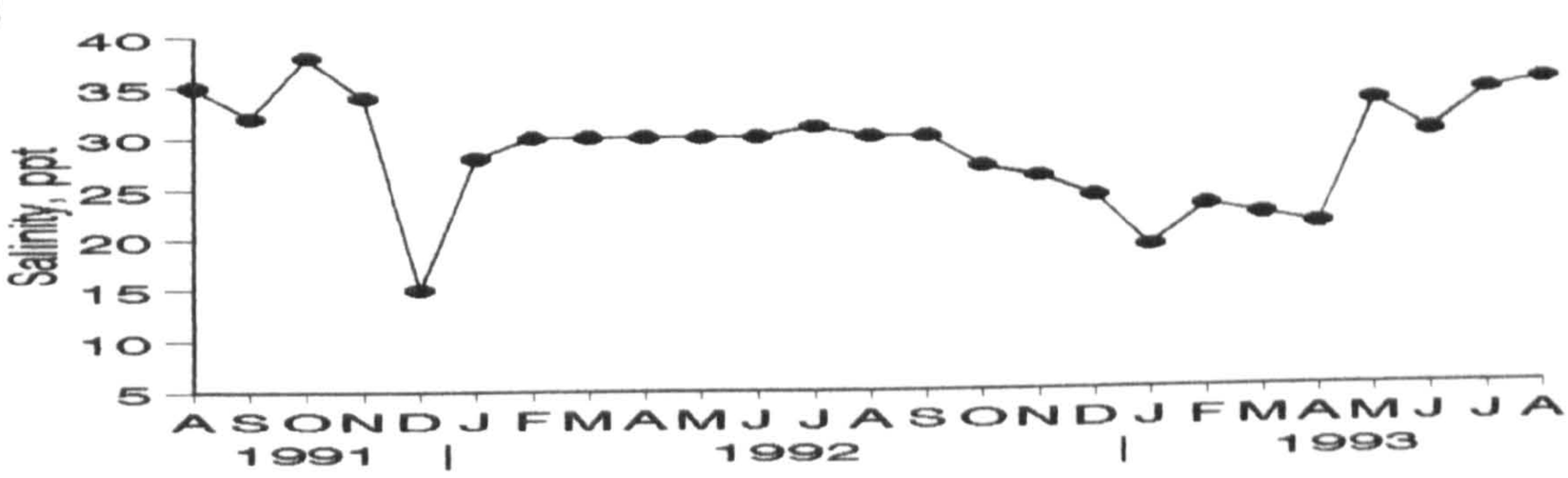
E. The seasonal variation of surface sea water concentration of chlorophyll-*a*. Each point is the mean ± Standard Error of three readings.

F. The total monthly rainfall in Semarang, the nearest city to Tapak

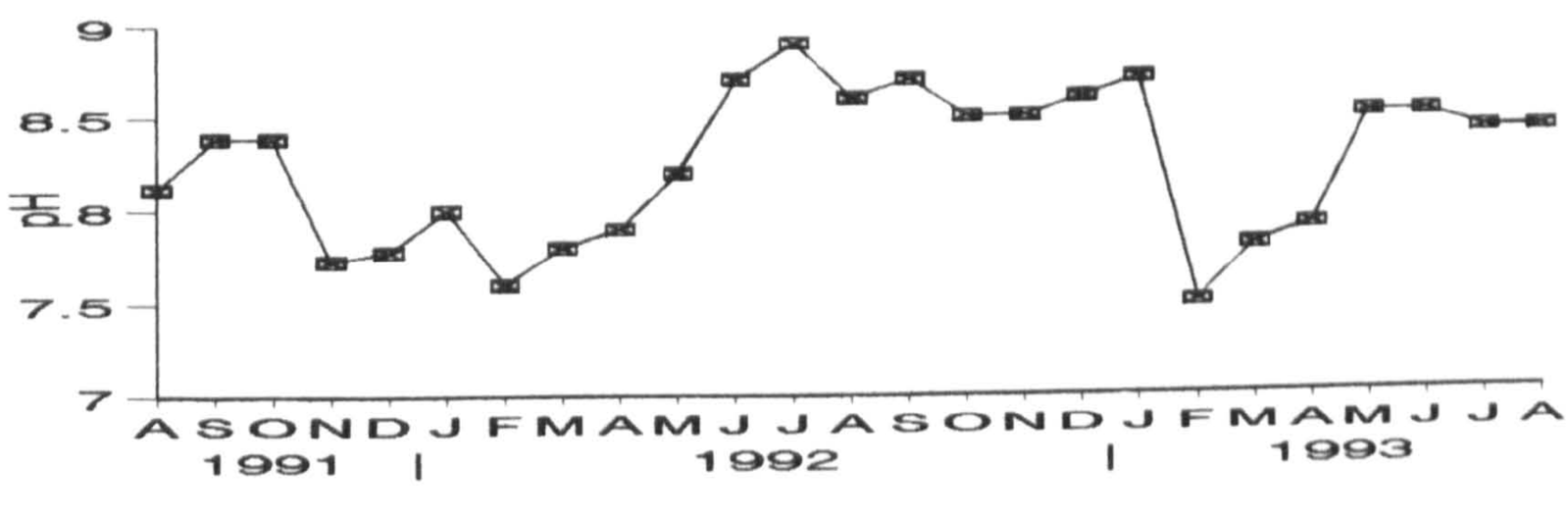
A


Temperature, °C
40
38
36
34
32
30
28
26
24
Water
Sediment
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

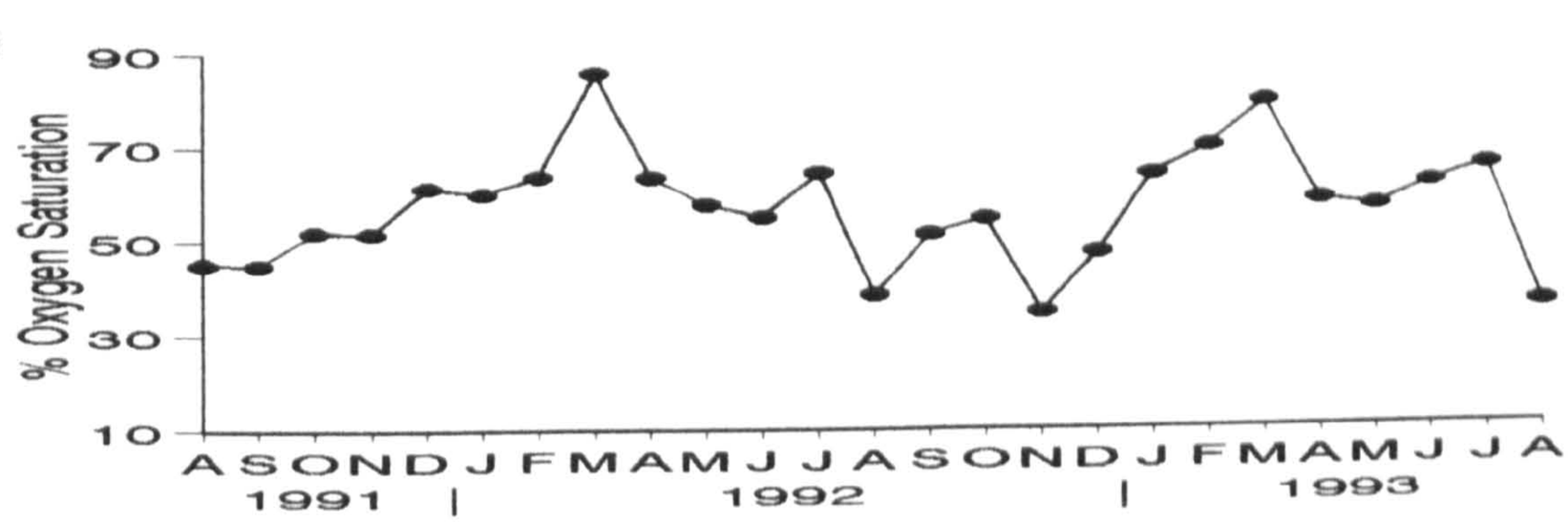
B


Salinity, ppt
40
35
30
25
20
15
10
5
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

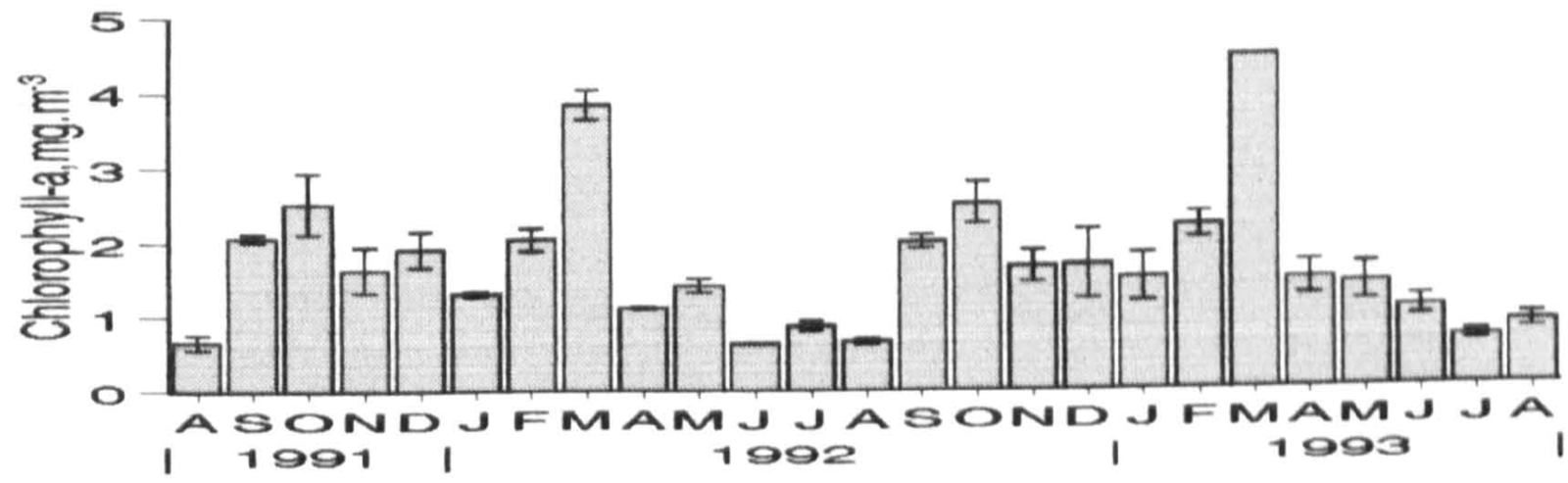
C


pH
9
8.5
8
7.5
7
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

D


% Oxygen Saturation
90
70
50
30
10
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

E


Chlorophyll-a, mg.m-3
5
4
3
2
1
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

F

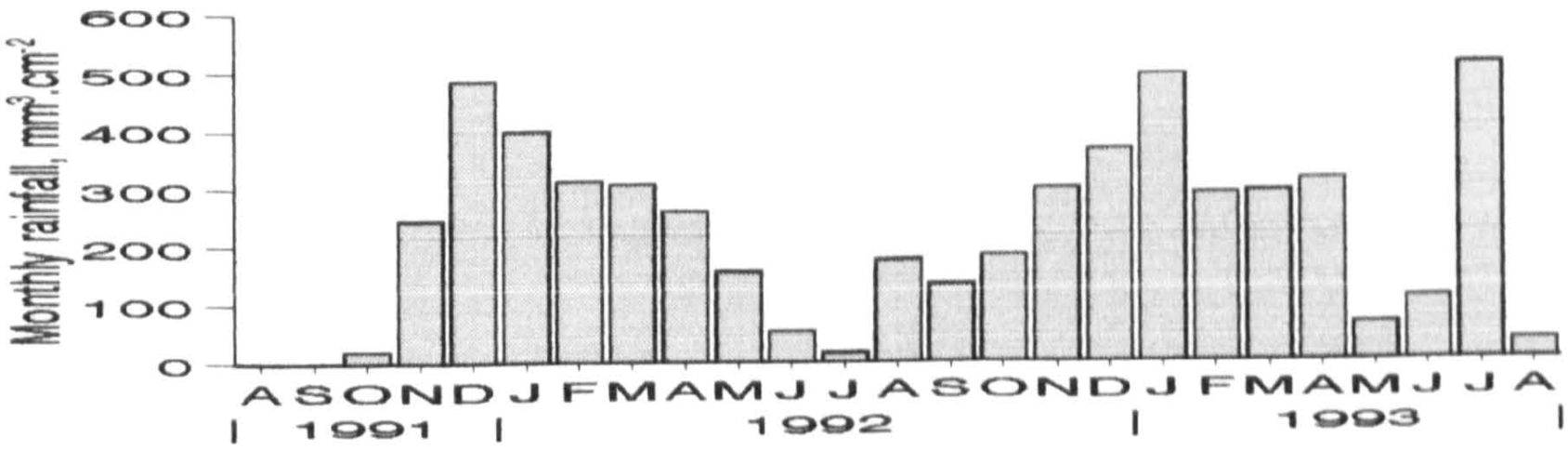

Monthly raintall, mm3.cm-2
600
500
400
300
200
100
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

Figure 9: Seasonal variability of some physical parameters in Bandengan during the study period August 1991 to August 1993.

A. Surface sea water (■) and sediment temperature (☒)

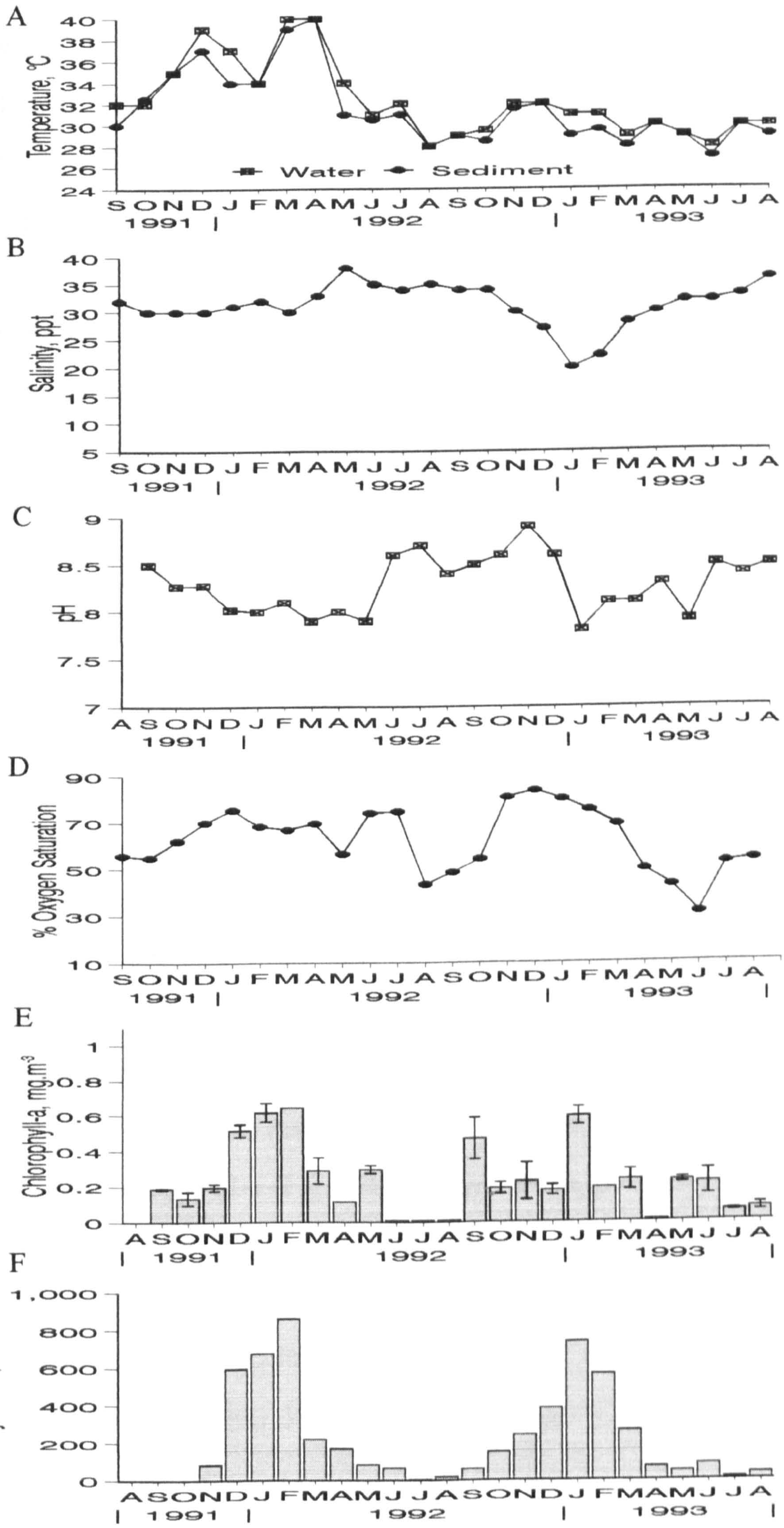
B. The profile of surface sea water salinity (●)

C. The pH (☒) of surface sea water

D. The seasonal variation of percentage oxygen saturation (●)

E. The seasonal variation of surface sea water concentration of chlorophyll-*a*. Each point is the mean ± Standard Error of three readings.

F. The total monthly rainfall in Jepara, the nearest town to Bandengan.


A
Temperature, °C
40
38
36
34
32
30
28
26
24
Water
Sediment
S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
Salinity, ppt
40
35
30
25
20
15
10
5
S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
C
pH
9
8.5
8
7.5
7
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
D
% Oxygen Saturation
90
70
50
30
10
S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
E
Chlorophyll-a, mg.m-3
1
0.8
0.6
0.4
0.2
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
F
Monthly Rainfall, mm3.cm-2
1,000
800
600
400
200
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

In this study, the percentage of oxygen saturation varied between 57.25% to 65.47%, with Tapak recording the lowest values. This decline in oxygen concentration at Tapak, mirrors the fact that Tapak estuary receives untreated waste in the form of run-off from the industrial areas a little further upstream. In general, the dissolved oxygen profile has two peaks (Figs. 7D, 8D & 9D) with the lowest levels occurring approximately at the pre-monsoon period (August-September) when salinity was relatively high, probably due to a high rate of evaporation. During the rainy season (December and January), dilution of sea water by saturated rain water increased the oxygen concentration.

Although the geographical features of the study sites are somewhat different, it appears that over the two year period of the investigation from August 1991-August 1993, some of the physical characteristics such as surface water and sediment temperature, pH, oxygen solubility and the dissolved oxygen values, did not differ significantly from one site to another as indicated by the average values and the standard deviation (Table 5). These results, therefore, indicate that the three selected sites lie in a relatively similar microclimatic region. Yet, there are between-site differences e.g. in the amount of monthly rainfall, seasonal salinity fluctuation, nature of the bottom sediment and the concentration of chlorophyll-a.

The chlorophyll analysis reveals that Wedung and Tapak have a higher concentration of chlorophyll-a (Figs. 7E-8E) and hence, more phytoplankton than Bandengan water (Fig. 9E). In Wedung, it appears that the phytoplankton bloom in 1991, as indicated by an increase in chlorophyll-a content of the sea water, occurred during the peak of the rainy season. Meanwhile in the following year, 1992, the highest concentrations of

chlorophyll occurred at a similar time of the year, although in the pre-monsoon period showed an abrupt fall in concentration during September when the oxygen saturation suddenly decreased (September 1991-1992, May-August 1993; Figs. 7E-9E). However, in Tapak, the highest chlorophyll concentrations occurred some three months after the highest monthly rainfall (Figs. 8E-8F) but closely followed the pattern of oxygen saturation (Fig. 8D). Bandengan showed a much lower (five fold) concentration in chlorophyll-a which, like Wedung, showed a pattern related to the periods of high and low rainfall (Figs. 9E-9F) and closely followed the months of declining and rising oxygen saturation (Fig. 9D).

From Table 6 (A: Wedung), it is clear that water temperature, oxygen saturation, and chlorophyll-a concentration individually are negatively correlated with salinity. Rain has a pronounced effect in reducing salinity whilst at the same time increasing the sea water temperature and oxygen saturation values, which in turn enhanced phytoplankton growth. Furthermore, the negative direction and degree of correlation between water temperature and salinity also support these findings, and indicates that the warmer the water the lower the salinity, although the correlation is only significant for Wedung. According to Weiss's table of oxygen solubility (Parsons et al, 1984) - even though the relationships are not linear, the higher the temperature and salinity of the water, the less oxygen and other gasses can be dissolved. These variables are in fact interdependent, so neither of them can actually represent independency over the other.

The percentage of oxygen saturation at all three sites is negatively correlated with salinity and conforms to the Weiss postulate, but is positively correlated to sea water temperature. Sampling was always carried out during

Table 6: The Spearman rank order of correlation (r) between some environmental factors monitored in: A. Wedung, B. Tapak, August 1991-August 1993 and C. Bandengan, September 1991-August 1993

A. Wedung

Source Parameters	Salinity	Rainfall	Water Temp.	$\%O_2$ Saturation
Water Temperature	-0.663 ***	0.458 *		
% O_2 Saturation	-0.518 **	0.414 *	0.462 *	
Chlorophyll-a	-0.292 ns	0.557 **	0.283 ns	0.385 *

B. Tapak

Source Parameters	Salinity	Rainfall	Water Temp.	$\%O_2$ Saturation
Water Temperature	-0.203 ns	0.155 ns		
% O_2 Saturation	-0.287 ns	0.449 *	0.221 ns	
Chlorophyll-a	-0.319 ns	0.183 ns	0.295 ns	0.208 ns

C. Bandengan

Source Parameters	Salinity	Rainfall	Water Temp.	$\%O_2$ Saturation
Water Temperature	-0.182 ns	0.264 ns		
% O_2 Saturation	-0.458 *	0.485 *	0.587 **	
Chlorophyll-a	-0.293 ns	0.130 ns	0.314 ns	0.147 ns

Significance level : ns not significant
* significant at 0.05>P>0.01
** significant at 0.01>P>0.001
*** significant at P<0.001

Degree of freedom: 24 (Wedung and Tapak), 23 (Bandengan)

the day at low tide when warmer, i.e. less dense fresh water from the river floating on top of the sea water as it flows into the estuary, may explain this phenomenon. Water flowing into estuaries will convey large quantities of oxygenated water (McLusky, 1989). This warmer, less saline and more saturated fresh water then warms up the sea water whilst reducing its salinity. Figures 7-9 (D-E) illustrate that despite the presence of a temporary sea grass bed in Bandengan during June-July 1992 and July-August 1993, the oxygen saturation profile showed a positive correlation with monthly rainfall. Wedung is the only site receiving a large volume of warm, more saturated fresh water from the surrounding rivers, yet the monthly rainfall significantly correlates with the oxygen saturation values.

A comparatively low degree of negative correlation between salinity and surface sea water temperature for Bandengan (r= -0.182) indicated that at this site, regardless of the state of the tide, both the salinity and sea water temperature were less varied and hence, more constant environmental conditions probably occurred as no large rivers discharge onto the bed. A significant positive correlation between monthly rainfall and oxygen saturation values in all three sites (r = 0.414, 0.449 and 0.485, Table 6) implied that salinities in these localities are more seasonally than tidally influenced (compare Figs. 7 to 9 A, D and F). Nevertheless, the conclusion of this study should really be based on more frequent monitoring, including those of the important related physical parameters which are not included in this study, for example wind-induced vertical mixing, current flow, water circulation, or rate of evaporation.

In general, sea water pH at all three sites was relatively low during the rainy season but increased during the dry season (compare Figs. 7-9C & F).

Surface water temperatures were also higher during the rainy season and this is particularly obvious during 1991-1992 (see Figs.7-9A & F). Increase in temperature, and salinity, as well as pressure, although the latter is negligible for shallow water bodies like estuaries, influences the flow of gasses across the air/sea water interface and alters the dissociation constants of carbonic acid thus causing a fall in pH (Meadows & Campbell, 1987). In Wedung, a slight elevation in water temperature to 32- 36°C at the onset of the rainy season in 1992-1993 (compare Figs. 7A & F) may explain the sudden decrease in pH in between November and February. However, in Tapak and Bandengan, where the surface water temperature during the rainy season in 1992-1993 remained relatively low at 30-32°C, there was a decrease in the pH to 7.4, the lowest level during the period of study.

Fine sedimentary deposits of mud, are a characteristic feature of estuaries. The process of sedimentation is extremely complex and depends on the interplay of various factors including water movement, topography, the amount and type of available sediments. Whilst sedimentation is generally related to the seasonal input of fresh water, storms and floods can transport large amounts of sediment into estuaries on a very irregular basis, as in Wedung. The regular pattern of particle size composition of the sediment at Tapak changed after the extensive flooding during December 1992-January 1993 (Fig. 11A).

Analysis of sediment samples collected between February and May 1993 following flooding showed an increase in the silt, clay and fine sand component of the sediment. The results of particle size analyses are given in Figures 10-12, whilst an overall summary of the sediment characteristics from the three sites are shown in Table 5. It is clear from Table 5 that the

more exposed beach of Bandengan consists of coarser grades of sand than Tapak or Wedung, whilst the latter site has a greater proportion of clay and silt particles. The greater degree of wave action in an open beach like Bandengan prevents the settlement of finer material. Sheltered beaches like Tapak and Wedung tend to have more clay and silt fractions than the open beach at Bandengan. The relative absence of fine sand in Wedung is substituted by a very fine sand fraction which has a similar proportion at the other two sites. In Wedung, the correlation between the presence of clay and the percentage of moisture retained by the sediment sample during analysis, revealed that clay improves the water holding capacity of the sediment ($r = 0.657$, $P<0.001$), whereas the presence of the very fine sand fraction significantly reduced the water retention ability of the sediment, $r = -0.854$ at $P<0.001$ (Fig. 10). Both fractions are negatively related to each other ($r = -0.782$, $P<0.001$). Similarly in Tapak, fine and very fine sand decreased the amount of water held in the sediment ($r = -0.769$ and -0.597 at $P<0.001$ respectively, Fig. 11); again clay balanced the effect ($r = 0.581$, $P<0.01$). Figure 12 illustrates the importance of the clay fraction in the water sediment retention properties since its absence from the April 1993 sample from Bandengan reduced the water holding capacity of the sediment from ca. 25% to 18%. The correlation between fine and very fine sand ($r = -0.915$, $P<0.001$) as well as between silt and clay is negative ($r = -0.566$, $P<0.05$). The nature of well-sorted coarsely-graded sand provides better drainage in Bandengan, as less water could be held (25% on average) in the larger interstitial spaces of the coarser grained sand of this exposed sandy beach.

Figure 10: Seasonal variability of the median grain size of the bottom sediment in Wedung.

A. Cumulative percent of the four major constituents of the sediment fraction, i.e. fine sand (250-125μm), very fine sand (125-62.5μm), silt (31.2-3.2μm) and clay (1.9-0.12μm).

B - E: The relationship between percent weight of clay (B), silt (C), very fine sand (D) and fine sand grains (E) to the overall water content in the sediment quantified as moisture percentages. The percent moisture of the samples is shown directly above each figure.

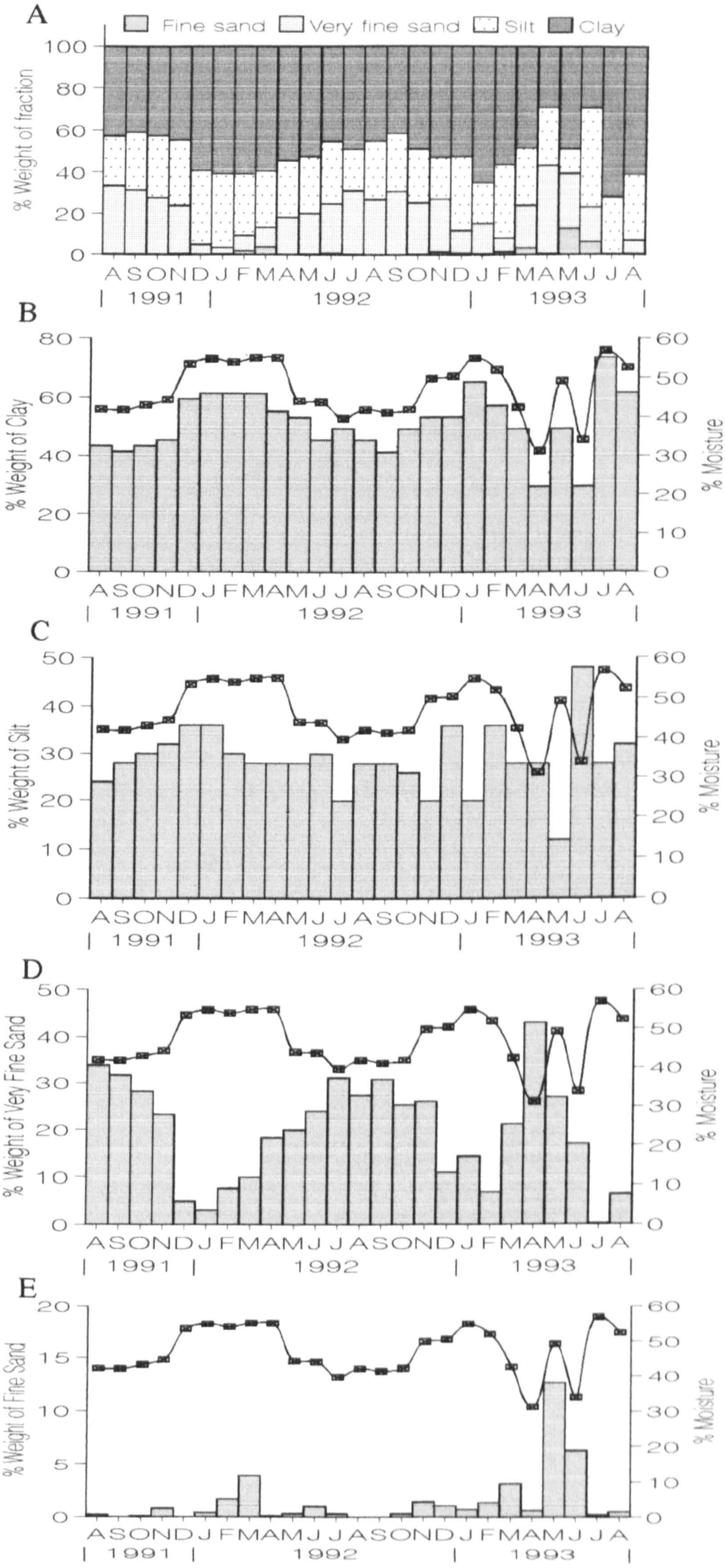

A
Fine sand
Very fine sand
Silt
Clay
% Weight of fraction
100
80
60
40
20
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
% Weight of Clay
% Moisture
C
% Weight of Silt
D
% Weight of Very Fine Sand
E
% Weight of Fine Sand

Figure 11: Seasonal variability of the median grain size of the bottom sediment in Tapak.

A. Cumulative percent of the four major constituents of the sediment fraction, i.e. fine sand (250-125μm), very fine sand (125-62.5μm), silt (31.2-3.2μm) and clay (1.9-0.12μm).

B - E: The relationship between percent weight of clay (B), silt (C), very fine sand (D) and fine sand grains (E) to the overall water content in the sediment quantified as moisture percentages. The percent moisture of the samples is shown directly above each figure.

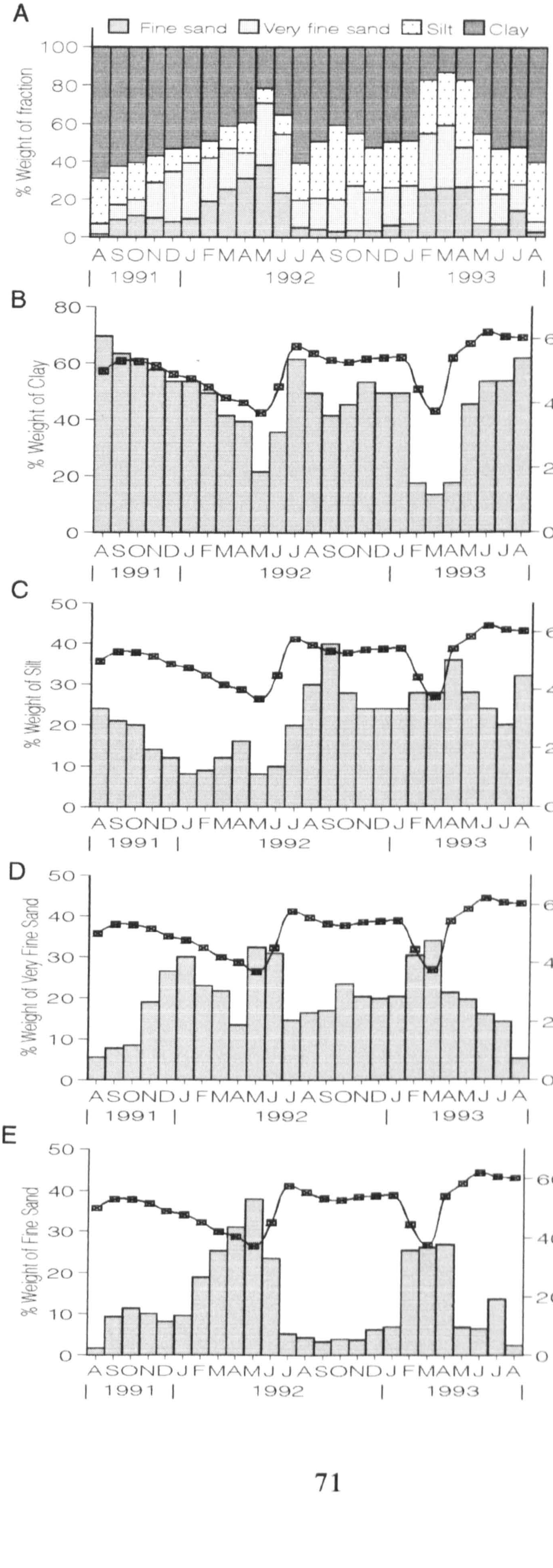
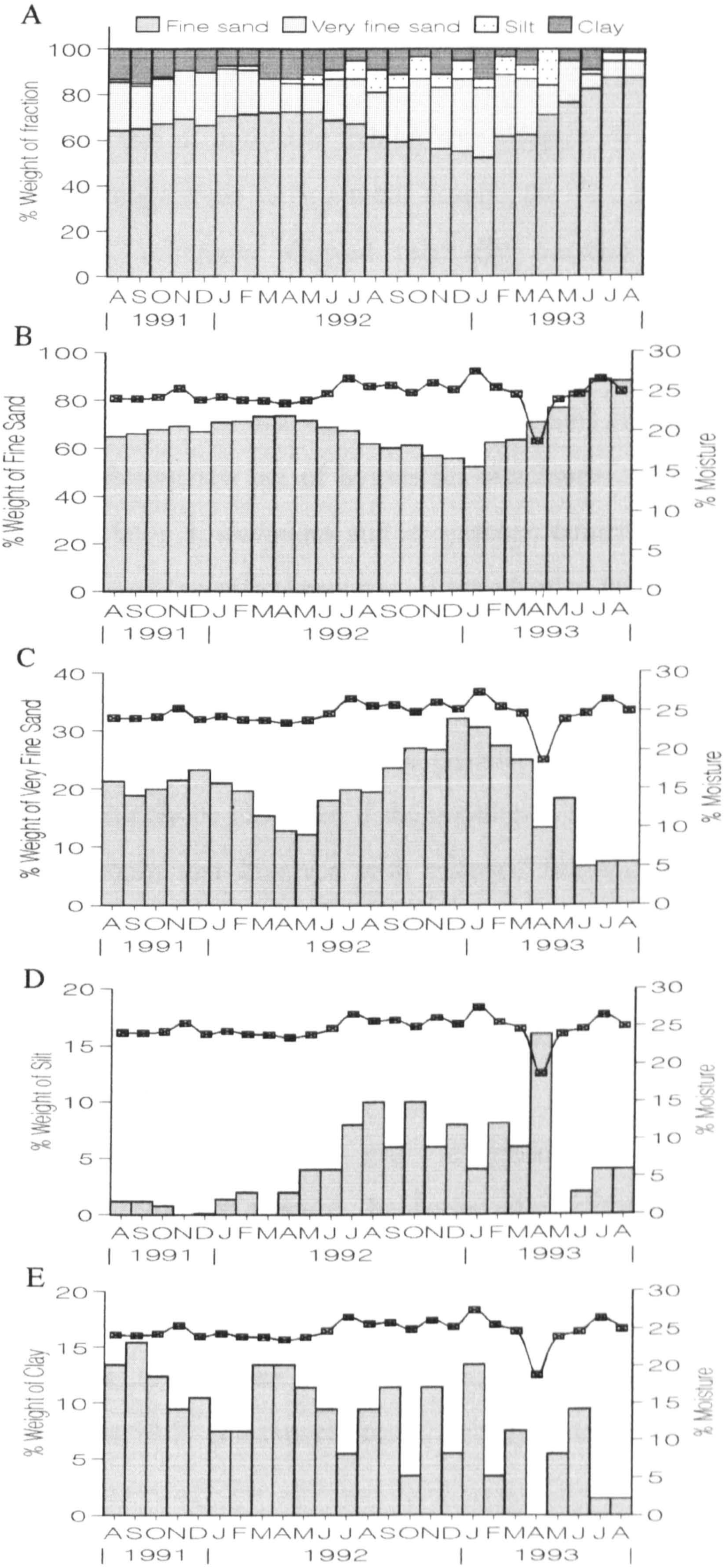

A
Fine sand
Very fine sand
Silt
Clay
% Weight of fraction
100
80
60
40
20
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
% Weight of Clay
% Moisture
C
% Weight of Silt
D
% Weight of Very Fine Sand
E
% Weight of Fine Sand

Figure 12: Seasonal variability of the median grain size of the bottom sediment in Bandengan.

A. Cumulative percent of the four major constituents of the sediment fraction, i.e. fine sand (250-125μm), very fine sand (125-62.5μm), silt (31.2-3.2μm) and clay (1.9-0.12μm).

B - E: The relationship between percent weight of clay (B), silt (C), very fine sand (D) and fine sand grains (E) to the overall water content in the sediment quantified as moisture percentages. The percent moisture of the samples is shown directly above each figure.


A
Fine sand
Very fine sand
Silt
Clay
% Weight of fraction
100
80
60
40
20
0
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
% Weight of Fine Sand
% Moisture
C
% Weight of Very Fine Sand
D
% Weight of Silt
E
% Weight of Clay

2.4. Discussion

Both *A. granosa* and *A. antiquata* exhibit some degree of aggregation within the largely homogeneous environment despite the fact that average densities are low. *A. antiquata* showed relatively constant figures of dispersion indices, suggesting they are more sedentary so once settled they would be less likely to move within or from the more stable structure of the bottom sediment. Even so, when dislodged these blood clams are capable of re-burrowing and producing new set of byssus for attachment. The highest level of dispersion in both *A. antiquata* and *A. granosa* coincides with the peak spawning season and juvenile recruitment. Reproductive behaviour may be the factor responsible for the existence of these patterns. It is clearly in the interest of any species to reproduce successfully and the chances of a successful encounter between male and female gametes released directly to the water would presumably be increased if the population is clumped. Such a viewpoint would imply that bivalves with external fertilisation should exhibit patchiness in their pattern of distribution. Hughes (1970), however, found that a population of *Scrobicularia plana* from a temperate shore was generally randomly distributed, yet there was some evidence for an overall patchy distribution and randomness within clumps.

In the mudflats in West Malaysia, *A. granosa* appear to be very highly aggregated (Broom, 1982b). On average, the degree of aggregation of both *A. granosa* in Wedung and *A. antiquata* in Bandengan was low (Id= 1.35 and 1.63 respectively) compared to that shown by a population of *A. granosa* in West Malaysia (Broom, 1982b). The average of the dispersion index for the Malaysian population is 50.57 and ranged from 2.5 (only in one occassion in February 1977) to 219.

On the west coast of Malaysia *A. granosa* is often found at highest densities on mudflats close to but not within, estuaries and mouths of tidal mangrove creeks (Pathansali, 1963; Broom, 1985). This characteristic habitat is similar to where the monthly samples for this study were regularly collected. Broom (1982b) reported the population densities of 33-63 indiv.m^{-2} . Instead, the relative abundance of the populations in Wedung was much lower, i.e. 9.41 ind.m^{-2}. With a range from 0.0-30.85 ind.m^{-2} the population density in Wedung is somewhere in between the range reported in Thailand (Boonruang and Janekarn, 1983), i.e. 0.06-2.2 ind.m^{-2} and the high density in West Malaysia. Even so, the highest densities in Wedung are still well below those recorded by Broom (1982b).

Studying animal-sediment relationships in Buzzard Bay, Massachusetts, by theoretical considerations of the dynamic factors Sanders (1958) derived the value for the optimal median grain size for filter feeders as 180μm and also found that at stations where deposit feeders predominated, the silt-clay fractions showed uniformly high values (50-90%). Broom (1982b) reported that at two mudflats he studied for *A. granosa,* particles less than 53μm were between 80-90% (by weight). Yet the condition in Wedung and Tapak are not in the closest proximity to those fractions, since the average silt-clay fraction as percent weight were 59.73 ± 25.68% and 59.12 ± 52.17% respectively. Further comparison of the study sites with those reported in Boonruang and Janekarn (1983) revealed that mudflats in the study sites in Thailand comprise 18.16 to 29% mud (determined as 63μm grain size) by total weight. The water content of these substrata in Thailand (47-51%), Wedung and Tapak (48-51%) reflected their predominantly sandy nature. Whilst commercial harvesting may help to account for the low densities in

Wedung and Thailand, it seems likely that the rather sandy substratum in Wedung, Tapak and Thailand represents a suboptimal habitat for this species.

The mesogastropod *Natica maculosa* and a neogastropod, *Thais carinifera* have been identified as potential predators of *A. granosa* populations in West Malaysia (Pathansali and Soong, 1958; Bardach et al, 1972; Broom, 1981, 1983c). Broom (1983c) demonstrated that at different densities (125 to 2,500 indiv.m^{-2}), there was no evidence of a difference in mortality rates. However, his experiments did reveal distinct differences in mortality rates at different shore elevations, with mortality likely to be greatest at low shore levels. The author ascribed this to the relatively greater degree of accessibility of low shore populations to aquatic predators, a conclusion borne out by the greater preponderance of drilled shells found at lower elevations. These predatory gastropods *N. maculosa*, *N. tigrina* and *T. carinifera* were found to be present in abundance subtidally offshore. However, samples of *A. granosa* collected subtidally from Wedung and Tapak were never found to have drilled shells characteristic of predation by the moon shell *N. maculosa*. In fact, dead assemblages of bivalve shells were rarely encountered from these areas. Nevertheless, *A. granosa* is not the only prey species available to these two predators in the field (Berry, 1982, 1983). Similarly in Bandengan, although some *N. maculosa* were occasionally collected, it did not appear to be preying upon *A. antiquata*.

Broom (1985) demonstrated that the degree of infestation of clam shells by *Balanus amphitrite* tended to be a function of aerial exposure. The highest percentage of blood clams supporting-barnacles (7.22%-13.93%) were found living between 110-200 cm above CD (Chart Datum). This declined (0.82 to 2.23%) in clams living 230-250 cm above CD. In agreement with Broom

(1985), at high water of neaps in Wedung and Tapak water depth can be more than 3m subtidally and such barnacle encrustation was negligible (0.054%). Only clams placed in cages half burried in the sediment for 49 days in tambak at ca. 100 cm depth at high water of neap tides suffered a high infestation of barnacles.

To some extent, Wedung and Tapak show a certain similarity in the composition of their benthic communities, but the species differ from those found at Bandengan. It can be seen from Tables 2-4 that Wedung and Tapak are dominated by 19 and 18 bivalves species respectively, 11 species of which are found in both sites; in addition, 3 out of 6 species are gastropods. The shore in Bandengan is predominated by 14 gastropod and 11 bivalve species, 1 species of sea cucumber and 5 species of crabs. The last two groups are characterised by having only a few species but a high number of organisms. By comparison, in a west Malaysian mudflat there were 22 macrobenthic species, i.e. 8 species of bivalves, 11 gastropod species, 1 scaphopod and 2 species of crustaceans (Broom, 1982b). In Thailand, Boonruang and Janekarn (1983) described the animal community in each of three zones on the shore of Sapum Bay, Phuket, which were inhabited by *A. granosa* and found a total of 58 macrobenthic species.

The presence of a large population of Pacific oysters *Crassostrea gigas* in Tapak may have valuable role to play in increasing the productivity of the area in which they live by filtering out food from the water column above them and depositing material as faeces and pseudofaeces which in turn is available to benthic organisms. McLusky (1989) reported that *Crassotrea gigas* can deposit material on the bottom, equal to that resulting from the combined activity of plankton and gravitational sedimentation in a 45m

column of water. The commonly present sea cucumber *Holothuria leucospilota* as sediment re-workers in Bandengan may be comparable in their biodeposition role as *Crassostrea gigas* in Tapak.

Kasigwa and Mahika (1991) reported that *A. antiquata* is strictly phytophagous. They found 27 phytoplankton genera in the digestive tracts of their specimens, but never found any specimens of zooplankton. The present study observed the presence of remnants of foraminiferan in the gut of *A. granosa* and *A. antiquata*, besides large quantities of benthic diatoms typically found in coastal waters (see also Broom,1985). Both these two bivalve species presumably extract their food both from the water column as well as from the water-sediment interface. So, it may be misleading to classify organisms rigidly based on their trophic status since there has been increasing evidence that deposit feeders may operate as suspension feeders and vice versa (Wade, 1972; Eagle and Hardiman, 1977; Peterson, 1977; Maurer et al, 1979; Broom, 1982b). These studies suggested that suspension feeders may utilize benthic diatoms and microalgae, resuspended matter and associated bacteria so that the distinction between suspension feeders and deposit feeders becomes blurred.

The most interesting aspect of the results presented above is the evidence for the existence of a marked seasonality amongst the environmental factors monitored. McLusky (1989) stated that in most estuaries there exists a close connection between sea water salinity and type of substratum, where reduced salinity is associated with finer sediment particles. Table 5 showed the coincidence between such an association. On average, Wedung has the lowest sea water salinity and the highest clay content. This was followed in order by Tapak and Bandengan. At Perak and

Penang, Malaysia, it was noted that during the dry seasons when rainfall is below average, the salinity of the coastal waters around the *A. granosa* beds usually varied between 26-31‰ (Pathansali, 1963), whilst in the rainy season the salinity over some blood clam beds may drop to as low as 5-10‰ during low water of neap tides, or as low as 15‰ at high water (Broom, 1985). However, exceptionally low values do not usually persist over natural beds for more than one or two weeks. Seasonally, salinities were in the range 18.2‰ to 25.9‰. The conditions are similar with those in Tapak and Wedung although here there is a greater range, 15-38‰ and 6-40‰ respectively.

Large scale mortality of *A. granosa* during the rainy season is not unusual and has been reported from time to time. For example in Wellesley Province, Malaysia, mass mortalities (20-50%) of *A. granosa* were attributed to a period of particularly heavy rainfall when salinities in almost all of the estuaries studied remained between 0 and 7‰ for a period of at least 14 days (Liong, 1979). Pathansali and Soong (1958) noted that on culture beds of *A. granosa* in Penang and Perak, Malaysia, a mortality of 80% could be experienced between sowing and harvest, i.e. 10-12 months. However, Broom (1983c) recorded no massive mortality of *A. granosa* during the wet season in the populations he studied, but blood clams were mainly established outside the mouth of the river Selangor. Moreover, he never recorded a salinity less than 14.0‰ over artificially seeded beds (Broom, 1980).

During the two year period of this study, there were ocassions in Wedung when the salinity dropped to well below 14‰ (e.g. 7 and 6‰ in December 1991, 1992). On the second occasion, the decrease in salinity

began in October 1992 and remained below average until March 1993 when it returned to the normal level following a big flood in February 1993 (Fig. 7B). However, neither small specimens nor death assemblages could be found. Thus it is difficult to distinguish whether their absence was because of mortality due to the sudden drop of salinity or because they were flushed down shore into the subtidal by the out flow from the rivers. The latter is a more reasonable suggestion because after this flood, a number of the local fishermen trawled spat for sowing in the tambaks from the subtidal areas. Pathansali (1963) found that early spat of 0.25-1.10mm were more tolerant and more active (based on opening rates and movement) over a wider range of salinity than larger clams.

Only after a severe flood for more than 10 days in December 1992 did the blood clams suffer. On this occasion layers of very fine mud were deposited about 3mm around the mantle edge and the animals easily bled on exposure to air. Their poor condition may have been compounded at that particular time because they had just spawned (see Chapter III). During a second flood in February 1993 the clams did appear to be in their normal condition after the flood water had receeded. Surprisingly, *A. antiquata* did not display the kind of poor condition shown by *A. granosa*, although they too had spawned in November 1992. For *A. antiquata*, Toral-Barza and Gomez (1985) reported a salinity range of 27-36‰ in the Philippines during their study period, whereas in Tanzania the range was quite narrow, only 34-35‰ (Kayombo and Mainoya, 1987).

Laboratory experiments have confirmed that *A. granosa* is an osmoconformer (Davenport and Wong, 1986), which responds behaviourally to low salinity (≤19‰) by tight and effective valve closure. However, Patel

and Eapen (1989a) working on *A. granosa* collected from Bombay waters, India, maintained that under laboratory condition, clams exposed to reduced salinity (18‰) will continue to exhibit normal behaviour by opening and closing their valves at least four times during a 20 min period. Only on exposure to a further reduction in salinity (9‰) did the same groups show a quick response by closing their valves. Djangmah et al (1979) studied the response of *A. senilis* to changes in salinity and found that the valves remained open at salinities down to 15‰. Similarly, below this salinity, the clam isolated itself from the environment by closing up.

Other species of *Anadara*, e.g. *A. granosa bisenensis*, *A. subcrenata*, *A. tuberculosa*, *A. senilis*, appear to have similar preferences for areas with an estuarine influence (Cahn 1951; Ting et al, 1972; Squires et al, 1975; Okera 1976). Yankson (1982) however, recorded the presence of a population of *A. senilis* in a Ghanaian lagoon which experienced an increase in salinity up to 50‰ during the dry season and Baquiero (1980) found that populations of *A. tuberculosa* in Baja California Sur, Mexico, were subjected to salinities in the range of 30-40‰. Yet the mode of tolerance of species of *Anadara* to large fluctuations in salinity is not clearly known.

The temperatures to which species of *Anadara* are exposed vary according to their geographical range. During periods of minimal water movement subpopulations high on the shore may be subjected to considerably high temperatures. Temperature experienced by *A. granosa* in Malaysia are generally in the region of 29 to 32°C (Broom, 1982b) or 25 to 32.8°C in Thailand (Boonruang & Janekarn,1983). Under neap tide conditions, the temperature at the water's edge on a sunny day could rise to 40°C and the temperature of the mud flat may drop several degrees during

the night, or even fall as low as 25°C in the early morning (McIntosh, 1978; Broom, 1985). In comparison to those records, populations of *A. granosa* in this study encountered a wider sediment temperature range, i.e. 26-36°C in Wedung and 28-39°C in Tapak. A population of *A. antiquata* in Calatagan, Philippines, experienced a temperature range from 26 to 33°C (Toral-Barza and Gomez, 1985). In Bandengan, sea water temperatures ranged from 27-40 °C. In Tanzania, in which another population of *A. antiquata* was studied, no data are available for sea water temperatures; however, the average daily air temperature ranged from 18 - 34°C (Kayombo and Mainoya, 1987).

Other tropical bivalve species survive temperature regimes similar to those experienced by *A. granosa*. For instance, Squires et al (1975) noted that temperatures in the mud inhabited by *A. tuberculosa* in Colombia ranged from 26 to 37.5°C, or 29.5 to 35°C within the mangroves swamps in Baja California Sur, Mexico (Baquiero, 1980). Yankson (1982) mentioned that in some lagoons in Ghana inhabited by populations of *A. senilis*, temperatures may be consistently in the region of 32 to 34°C. *A. broughtoni* and *A. subcrenata*, being inhabitants of temperate waters, are subject to a much wider range of temperatures, from 6°C in February to 27°C in September (Ting et al, 1972).

In the natural habitat *A. granosa* is subject to fluctuations in oxygen tension of sea water. On the flood tide, the oxygen content of seawater as it rises over a mudflat could be reduced from saturation to less than 60% saturation. Moreover, the temperature of the standing water on the mudflat could rise considerably during the day, thus further contributing to a reduction in oxygen content of the water. In this study, the percentage of oxygen saturation was between 57-65%. The average dissolved oxygen

lower (2.48 to 2.90mg.l^{-1}) to that reported by Boonruang and Janekarn (1983) in Thailand (6.6±0.7mg.l^{-1}), or Liong (1979) in Malaysia (4.03±1.41mg.l^{-1}) where the range of 2.53-7.00mg.l^{-1} coupled with depressed salinity proved to be detrimental to *A. granosa*.

The genus *Anadara*, unlike most other bivalve species, is known to contain haemoglobin in the erythrocytes of the blood. Laboratory experiments have confirmed that *A. granosa* is an osmoconformer (Bayne 1973; Davenport and Wong, 1986; Rao et al, 1990) and is tolerant of long periods of low oxygen content as well as temperature fluctuation (Collett and O'Gower, 1972; Bayne, 1973; Patel and Eapen, 1989a). It is also capable of taking up oxygen at a similar rate in both air and water (Davenport and Wong, 1986). This finding is unusual amongst intertidal animals, most of which exhibit a reduced oxygen uptake in air; even such well adapted forms as *Cerastoderma edule* (Boyden, 1972).

Most bivalve molluscs respond to aerial exposure by closing their shell valves completely or by maintaining a very narrow gape which minimises water loss while permitting gaseous exchange. *A. granosa* and *A. antiquata* exhibit different responses to such conditions. Animals taken from water and kept at room temperature gaped widely within ca. 10 minutes presumably to exploit the rich source of oxygen available in air. At least 70% were observed to be gaping until more than 6 hours had elapsed. Thereafter, clams progressively closed and the first deaths were recorded between 36-48 hours. It is possible that the resistance of the Anadarinae to low oxygen tensions is aided by the fact that they contain haemoglobin and erythrocytes in their haemolymph (Broom, 1985; Davenport & Wong, 1986). *Anadara* (=*Senilia*)

senilis, previously the sole representative of the genus *Senilia*, is also reported as an oxygen regulator at oxygen tensions between 50% to 100% saturation (Djangmah et al, 1979).

Phytoplankton species are an integral part of the estuarine ecosystem but, high turbidity and distinctive patterns of water circulation clearly reduce phytoplankton growth in many estuaries (Boaden & Seed, 1985). Other factors that may limit the production of phytoplankton in estuaries is the depth of the water. The shallow nature of an estuary may mean that the mean depth of water is less than the optimum depth for producing maximum net photosynthesis. For example in a 400km^2 system of estuaries near Beaufort, North Carolina, a mean depth of water at 1.18m was less than the optimum depth of 1.7m for maximum net photosynthesis (McLusky, 1989).

Estimates of chlorophyll-a concentrations presented in Table 5 and Figures 7D-9D show that the highest concentrations in Wedung were still below the concentrations in the sea water above a densely populated *A. granosa* bed in West Malaysia (7.74 ± 2.16mg.m^{-3}; Broom, 1982c) and well below chlorophyll concentrations in adjacent areas where blood clams were absent, i.e. 17.4 ± 2.90mg.m^{-3}. This feature may be attributed to the different depths from where the samples were obtained. Compared to sampling 60-100 cm above the sediment in this present study, Broom (1982c) apparently collected his samples from a water column 275-350 cm above CD. Nevertheless, his finding of higher concentrations of chlorophyll-a should probably be treated with caution since, due to the interference from detrital chlorophyll, the technique used by Broom (1982c) was recommended only for waters near the surface euphotic zone (Lorenzen and Jeffrey, 1980). Sea water samples collected by Broom (1982c) were obtained using a diver with

a snorkel, each bottle being carried down by the diver and filled from within 5 cm of the bottom sediment.

Rainfall clearly has a pronounced effect particularly in determining the salinity profile of the water bodies. The salinity profile in Wedung, Tapak and Bandengan are seasonally influenced. However, from the chlorophyll-a concentration and granulometry of the bottom sediment the physical conditions in Tapak appeared to be somewhere between Wedung and Bandengan. Moreover, considering the relationships between temperature, salinity and oxygen saturation profile, salinity in Tapak is influenced by both the tide and relatively continuous rainfall over the years. The latter contributes in aerating the water body (yet the oxygen concentration in Tapak is still the lowest) which in turn induces phytoplankton growth, at the same time diluting and compensating for the effects of pollution from untreated industrial sewage flowing from upstream.

From this chapter, useful information regarding the characteristic habitat of natural populations of *A. granosa* and *A. antiquata* may be summarised as follows. Members of the anadarinids may occur in such contrasting habitats ranged from exposed sandy shores to sheltered mudflats in front of mangroves. Even so, those inhabiting the latter habitat cannot be considered as typical mangrove inhabitants because they can and do appear elsewhere. For example, *A. granosa*, *Cunearca pilula* and *Scapharca cornea* can also be observed subtidally.

Sharing similar habitat with *A. antiquata*, other genera within the family Arcidae, e.g. *Barbatia spp.* is typically somewhat heteromyarian in form broadly resembling a mussel but smaller in size than for example *Arca sp.* which is much larger. Both genera have a wider byssal notch to

accommodate a massive byssus and usually they are approximately equivalve. All of these features can be thought of as adaptive features assosiated with inhabiting such an exposed area.

Being relatively sheltered estuarines, Wedung and Tapak are characterised by fine sediments and therefore have a higher interstitial water content, lower salinity values and higher concentration of chlorophyll-a. This represents a relatively stable environment and contains a higher biodiversity with the highest macrofaunal abundances predominated by bivalves and crustaceans including brachyuran decapods.

By comparison, Bandengan is a relatively open beach with a few small streams flowing onto the beach, and is characterised by a coarse gravel to fine sand which results in a low interstitial water content in the sediment, comparably high values of oxygen availability in the surrounding water but low levels of chlorophyll-a. The site exhibits a lower biodiversity in macrobenthic fauna which is composed mainly of mobile epibenthic species like gastropods and brachyuran decapods. The highest values of salinity and coarser sediment in Bandengan however, is attributed more to the igneous type and nature of the petrological facies close by in Jepara rather than to the salt flocculation processes common in the estuaries.

The fact that Tapak exhibits comparatively high biodiversity, and shows the highest diversity among the three sites studied, is probably the result of continuous rainfall. Besides the plentiful rainfall, the presence of large numbers of Pacific oysters *Crassostrea gigas* may help to improve biodeposition leading to a better food recycling. The regular presence of so called second and third grades of key species for clean flats in Tapak, i.e. *Meretrix lamarckii* and *Tachypleus gigas* (Morton and Morton, 1983),

suggest that the environment may self purify itself and therefore is capable of supporting such a diverse benthic community.

The low occurrence of *A. granosa* in Tapak was then attributed more to the predation encountered coupled with the rather sandy substrata which represents a sub-optimal habitat for this species, than as a result of chronically polluted environment. Both the populations of *A. granosa* at Wedung and *A. antiquata* at Bandengan appear to have no detrimental ectoparasites nor suffered a heavy predatory pressure. Harvesting for human consumption, however, is obviously the major factor diminishing the population of *A. granosa* in Wedung and *A. antiquata* in Bandengan. Severe environmental conditions such as flooding may also be deleterious; whereas crab predation, is more likely to occur than predation by gastropods, starfish or wading birds as in those populations reported in Thailand and Malaysia (Boonruang & Janekarn, 1983; Broom, 1982b).

Field observations in this study confirmed that *A. granosa* possesses remarkable physiological and behavioural adaptations to deal with short-term fluctuations in environmental conditions, e.g. tidal, salinity, temperature, high load of suspended matter/turbidity and reduced oxygen saturation. Although *A. granosa* typically colonised the mudflats in front of mangrove areas and extended subtidally, it also survived in the mangrove ecosystem higher in the tidal zone (tambaks). This adaptation to life in the landward margin of mangroves enables *A. granosa* to exploit a habitat not normally occupied by other bivalves.

By contrast, *A. antiquata* only occurred intertidally on the sandy substrata in a relatively open beach where there were less extreme environmental conditions apart from perhaps greater degree of wave action.

So far, the physiological mechanism underlying the tolerance of *Anadara* toward environmental fluctuations is thought to be the presence of haemoglobin which has been identified mainly for the anadarinids and *Solen legumen* living in muddy sand in the Mediterranean (Morton, 1963).

CHAPTER III

REPRODUCTION

3.1. Introduction

Many species of the genus *Anadara* are reported to be dioecious (Sullivan, 1960; Ting et al, 1972; Yoloye, 1974; Squires et al, 1975; Yankson, 1982). *A. granosa* and *A. antiquat*a have also been described by a number of authors as having separate sexes (Pathansali, 1964; Kastoro, 1978; Broom, 1983b; Toral-Barza and Gomez, 1985; Wong et al, 1985; Kayombo and Mainoya,1987). The wide zoogeographical distribution of the anadarinids makes them ideal for comparative studies. Many of the previous studies of this group have produced inconsistent findings with respect to the sexual characteristics of the species concerned; for example, the type of sexual development, sex ratio and occurrence of hermaphroditism, size at maturity or spawning periodicity were found to differ from one population to another.

Broom (1985) working on *A. granosa* in Malaysia, the only Southeast Asian country for which statistics on the culture of this particular species are available, found that there can be considerable year to year variability in the supply of spat. Therefore, a better understanding on the reproductive seasonality of *A. granosa* as well as for *A. antiquata* may help to explain such variations, which in turn should contribute to the basic fisheries data on how to sustain the natural broodstock.

An examination of the monthly samples of *A. antiquata*, occasionally revealed a small whitish unidentified pea crab (pinnotherid) within the mantle cavity of the clams. Pinnotherids are a group of brachyuran crabs adapted for life within other marine animals. They are distributed throughout subarctic,

temperate and tropical regions (Ruppert & Barnes, 1994). The occurrence of this crab and its effect on the physiological as well as on the reproductive condition of the clams is also documented in this chapter.

3.2. Materials and Methods

3.2.1. Treatment of samples of blood clams for histology preparation

Animals collected from each site were transfered into a large white collecting tray, from which 20-25 individuals of medium to large size were selected for histological examination of the gonad. Whenever small blood clams were available they were also included in order to determine the minimum size at which reproductive development first occurred.

Samples from Tapak almost always consisted of a few (<50) individuals, and therefore the number available for histology was reduced proportionately. On those occassions when <12 individuals were collected in any particular month, all blood clams were used for histological examination. No spawning occurred between the time of collection and the subsequent processing of the tissues (6-8 hours). Therefore it was felt that the fixed gonad state represented the condition at the time of collection.

Selected clams ranging from 15-40mm in shell length were opened at the hinge by cutting the adductor muscles. Following the routine protocols in Disbrey and Rack (1970), excess fluid was allowed to drain away and the whole tissue was separated from the shell, then fixed in saline (30%) Bouin's solution for 8 hours. After fixation, unwanted tissue was trimmed away and the main body mass rinsed with and stored in 70% ethanol. For each individual clam a piece of tissue was excised transversely through the body mass comprising the digestive gland, reproductive tissue and muscular foot, dehydrated using increasing concentrations of ethanol, then transfered to

xylene prior to embedding in paraffin wax (56°C MP). Sections were cut at 7 μm on a rotary microtome, and re-hydrated in descending ethanol series before staining in aqueous Haematoxylin and Eosin. As the mounting medium used is Canada balsam (DPX), which is non-aqueous, the sections were therefore, dehydrated and cleared in xylene. Following microscopic examination, stages in the reproductive cycle were photographed.

3.2.2. Assesment of gonad condition

Sexual maturation in lamellibranchs is usually divided into two stages: 1) development of the gonads from their primordia in young specimens, 2) seasonal development of gametes in adults, which is the main concern of this study. In practice, the best approach for monitoring changes in reproductive activity is to observe histological changes of the gonad tissue and then calculate such parameters as oocyte diameter, density and size distribution. Spawning can be detected by decreases in the density and proportion of mature oocytes. In order to quantify the changes of the male and female gonads during gametogenesis, a widely used scheme similar to that outlined for *Mytilus edulis*, *Modiolus modiolus* and *Cerastoderma edule* (Wilson & Seed, 1974; Seed & Brown, 1977a), *Mytilus edulis aoteanus* and *Aulocomya maoriana* (Kennedy, 1977), *Abra alba* and *Abra tenuis* (Nott, 1980) and for *A. antiquata* (Toral-Barza & Gomez, 1985) has been applied. Using this scheme, changes in the gonad can be classified into arbitrary stages according to the degree of development and the density of gametes. Gonad condition for both sexes was determined as follows:

1. Stage 1 developing (D1), follicles are mainly full of early stages of gametogonia, with only a few mature gametes. In females some further stages of development occur where the oogonia elongate and the micropyle

stalk becomes constricted. In this stage follicles have not attained their maximum size, a useful characteristic for distinguishing this stage from redevelopment stages where a new set of oogonia line the large and usually elongated half empty follicles.

2. Stage 2 developing (D2), male follicles are approximately half-full of mature sperm with several concentric layers of different stages of developing gametes. In females, approximately half the follicle is full of ripe oocytes, with some still in the primary stages of development. The blood cells sometimes occur in aggregations in the outer follicle wall.

3. Ripe stage (R), the follicles are completely full of mature sperm which are arranged with their acrosomes in centripetal position and their tails occupying the central position of the lumen. Although maximum density of ripe spermatozoa is attained, a thin layer of developing spermatogonia is still present. In females, ripe oocytes occur at maximum density and are arranged like bunches of grapes in the follicle lumen; they may take on a hexagonal shape as they are densely packed. Very few or no developing stages are present; the follicle wall becomes very thin and almost invisible.

4. Stage 2 spawning (S2), in the male follicle sperm can be seen streaming toward the centre of the lumen. In both sexes some empty spaces are left as sperm and oocytes are released. The shape of the follicles is clearly visible as these are now approximately half empty with only 1-2 layers of ripe oocytes/sperm.

5. Stage 1 spawning (S1), the lumen of male follicles either contains some residual sperm or is completely empty. Female follicles contain only a few residual rounded oocytes.

6. Stage 1 redeveloping (R1), after spawning, a broad layer of early gametogenic stages line the follicle walls. Follicles which are large and elongated in shape are less than half full of gametes.

7. Stage 2 redeveloping (R2), the follicles are approximately half full of developing and mature gametes.

8. Spent (S0), the lumen of the follicle is completely empty and occupies a minimum volume as it starts to collapse. No developing stages can be traced in the follicles or lining the follicle walls and therefore sexuality cannot be determined.

The sex of each clam in the monthly sample was also assessed visually from the external appearance of the reproductive tissue. This was done to detect any changes in sex ratio that might suggest sex change at a certain size of the clam. Any specimens whose sex could not be determined were considered to be 'undifferentiated'. However, when it became clear from the sections at what size gonads usually develop, any undifferentiated clams longer than 20 mm for *A. granosa* or 25 mm for *A. antiquata* were assumed to have spawned at least once or to be in the early stages of development as subsequently confirmed from the sections. The sex ratio of clams collected during the study period was calculated simply by dividing the number of males by the number of female specimens, and the goodness of fit of the model to 1:1 ratio tested.

3.2.3. Determination of gonad index

The gonad index, which provides an estimate of the reproductive condition of the population, was determined for both sexes of *A. granosa* and *A. antiquata*. The calculation follows the scheme by Toral-Barza and Gomez (1985) for *A. antiquata* adopted from the system proposed by Wilson and

Seed (1974) for *Mytilus edulis*. In calculating the gonad index, the eight gametogenic stages were merged into four categories and assigned numerical values as follows:

0 = spent
1 = stage 1 developing, spawning and redeveloping
2 = stage 2 developing, spawning and redeveloping
3 = ripe.

To calculate the mean gonad index, the number of clams in each stage was multiplied by the corresponding numerical value and the products averaged. Values close to 0 indicate the presence of inactive and/or spent individuals. Values close to 2 imply that spawning and/or development are occurring, whereas those approaching 3 denote the presence of ripe individuals. In general, an increase in the value of the gonad index implies that development of the gonad is proceeding, whilst a decrease implies that spawning is taking place. Nevertheless, this index cannot distinguish whether the clam is in the ripening or spawning stages because the index will be the same, i.e. 1 or 2. Therefore, annual gonad condition aggregated from both sexes in the subsamples and determined by means of this scheme as redeveloping, ripe, spawn, and spent was expressed in percentages to illustrate the seasonal variation.

3.2.4. The density and size distribution of oocytes

As an additional measure of gonad activity, in female clams the average density of oocytes and the diameter of oocytes in each monthly sample were also assessed. Tissue sections cut at 7μm were examined under a compound Leitz microscope fitted with an eyepiece graticule at x100 magnification. This unit was connected to a screen monitor. Based on 30 measurements, it was calculated that 1 division at magnification x100 ≈ 4μm ≈ 3.358mm on

the monitor screen; therefore 1mm screen ≈ 1.191μm. The screen is 275mmx200mm ≈ 78,042.66μm^2 ≈ 0.078mm^2 at magnification x100. For further analysis, all measurements were converted to a base of 1mm^2.

All measurements of oocyte diameters and densities were carried out on the screen and converted to an area equivalent to the field of view at a given magnification (i.e. x100). All oocytes encountered within selected areas of the sections were counted and measured. Point counts were made on 4 fields per individual female, i.e. in two regions at the left and right of the digestive tracts and another two at the dorsal portion of the body where reproductive tissues envelope the digestive glands. The data were transformed into percentages and the seasonal distribution of oocyte size plotted as histograms. The seasonal variation in oocyte density was plotted for the populations in Wedung, Tapak and Bandengan.

3.2.5. Assessment of the condition index, tissue weight, and pea crab infestation on *A. antiquata*

Whilst reproductive cyclicity of the subsample was studied histologically, another approach that proved useful in clarifying the seasonal changes in gonad maturation and spawning at the population level was the examination of the seasonal changes in dry tissue weight and the condition index of a standard-sized animal.

Many authors have used different methods for determining condition index, for example Squires et al (1975) used wet tissue/total wet weight for *A. tuberculosa*; Broom (1982b) applied the ratio of dry/wet tissue weight for *A. granosa*; Lee (1985) described two condition indices for *Perna viridis*, i.e. a) dry condition index: dry tissue/fresh whole body weight and b) wet condition index: fresh tissue/fresh whole body weight. Moreover, Wolff et al (1987) defined condition as ash-free dry weight (in gram) divided by the

product of shell length, shell height and shell width (in cm^3) for *A. senilis*, Kayombo and Mainoya (1987) described the condition index for *A. antiquata* as 1000x dry tissue/shell cavity volume.

Following Morton (1982) working on a fresh water bivalve *Corbicula cf. fluminalis*, one of the two most commonly applied condition factors was used to examine both size and site related differences in the condition of *A. granosa* from Wedung and Tapak. This formula was determined by Davenport and Chen (1987) as being the most statistically robust and involving stable, easy quantifiable parameters. The condition index (CI) was calculated as follows:

$$\text{C.I} = \left[\frac{\text{Dry Tissue Weight}}{\text{Shell weight}}\right] \times 100$$

The seasonal variation in the condition index for all monthly samples of *A. granosa* from Wedung and *A. antiquata* was plotted against shell length, and regression equations calculated along with the coefficient of correlation. This was also carried out when analysing the size related changes which relate the quantity of living tissue to the size of the animal. Dry tissue weight of the monthly samples was also analysed in the same way. An analysis of covariance with a single covariate (GLM in Minitab) was applied to examine any possible seasonal changes in population condition indices and to relate the findings to the histological data. The regression constants thus obtained from the monthly condition indices and shell length were applied to generate condition indices for 30mm standard-sized clams; the same set of analyses was carried out for dry tissue weight. This 30mm standard size was selected to represent the average size for the populations studied.

The correlation coefficients between condition index and shell length for *A. granosa*, produced from monthly samples, were used to observe the seasonal variation of these two growth parameters. Furthermore, in order to

observe the seasonal variation between overall physiological condition and the reproductive stages of the population, values of the condition indices acquired for 30 mm standard size animals were correlated with mean gonad indices for both populations of *A. granosa* and *A. antiquata*.

The degree and direction of correlation between population gonad indices and such environmental parameters as temperature, salinity and chlorophyll-a concentrations were also assessed. Similarly, but only for *A. granosa* (Wedung) condition indices of a 24mm standard size animal, as well as the gonad indices of both sexes were correlated to the fortnightly average of shell growth increments obtained from acetate peel replicas (details of preparation in Chapter IV). The values of these two growth parameters were plotted to examine changes between the somatic and reproductive growth throughout the year. This 24mm standard size was selected to represent the average size of the sectioned shells. Since the smallest individual with distinguishable reproductive tissues was 15.7mm and 23 mm for *A. granosa* and *A. antiquata* respectively, the 24mm and 30mm standard size animals presumably represented mature individuals.

In addition, an examination of the monthly samples of *A. antiquata* from Bandengan, occasionally revealed a small whitish unidentified pea crab (pinnotherid) within the mantle cavity. When present, the carapace width and sex of the crabs were recorded along with the shell length, sex, and whenever possible, the state of the reproductive tissue of the host. The sex ratio of male to female crabs was calculated. The degree of the occurrence of the crab against total numbers of individuals within monthly samples was plotted against the total number of all samples during the study period. The sex of the infested clams was determined from the external appearance of the reproductive tissues, whilst the relationships between size of the host clam

and size of male or female crab which occupied it was plotted and calculated. An analysis of covariance with a single covariate (occupancy by crab) was carried out upon dry tissue weight and shell length of comparable size ranges of infested and non infected clams.

3.3. Results

3.3.1. Gonad maturation and size at maturity

Examination of the histological preparations of both *A. granosa* and *A. antiquata* reveals no differences in the arrangement of the internal body organs. Also, no marked differences were noted between the two species regarding the colour of the gonad, final shape of the eggs or sperm, or the extent of the fully ripe gonad in relation to other organs. However, from the external appearance the relative sizes of the gonad are somewhat different. When ripe, the gonad of *A. granosa* always appeared to be less slender than that of *A. antiquata.* Regardless of the size of the individual animal, the proportion of tissue to shell in terms of condition indices is always lower in *A. antiquata* (Fig. 20). This is perhaps because *A. antiquata* has thicker blocks of muscular foot, so that the space left to accommodate the reproductive materials is less. The following descriptions apply to both species of *Anadara.*

Examination of the fresh visceral mass revealed that the gonad is situated in the basal region of the body, and to a varying extent envelops the dark green digestive gland. The male gonad is white and semiopaque whilst the female gonad is orange in colour. Haematoxylin gives a purplish colour to the sections. The main parts of the body such as the digestive glands, pedal muscles, gills, columnar epithelia of the digestive tracts and the reproductive tissues can be easily distinguished since they absorb the stain to various

degrees. The reproductive tissue consists of many ramifying tubules. At low power magnification cross sections of ripe male tubules are more rounded in shape, whereas the female gonad material in the tubules often appeared densely packed because the follicular wall is stretched and very thin. The earliest stage of gonad development could not be detected since throughout the year various stages of reproductive material were always present in almost all the specimens. A few individuals of both sexes were found in the fully spent stage, yet by the following month clams were already in a further stage of redevelopment. This suggests that there is no clear-cut seasonality in gamete maturation as shown in Figure 14. Similar findings for the Tapak population (Fig. 15) reveal that there is no cessation in the production of reproductive material throughout the year. *A. antiquata*, on the other hand, had a more seasonal reproductive cycle (Fig. 16).

Germ cell development is associated with a follicular system. Apparently, the primordial cells give rise to spermatogonia and oogonia. During oogenesis however, some observations (Plate 4A) reveal that the oogonia are initially flattened and attached to the follicle wall by the broad micropylar surface. As they grow, they become more elongated and basally constricted (Plate 4B). When the follicles approached maturity, the rapidly growing oogonia increase in volume. Finally the oocytes detach from the follicle wall and round off in the lumen. The nuclei were clearly visible in unfertilised oocytes. In this condition the visceral mass is distended with gametes which are readily visible through the thin body wall for *A. granosa*. In *A. antiquata* however, this ripe stage is less visible macroscopically due to their thicker body wall. After spawning (stage 2), follicles still contain few mature gametes. In both sexes gametogenic activity, which takes place from the undifferentiated cells lining the old follicles, may proceed simultaneously at

this stage thus making a quick transition to the active stage of redeveloping. At a later stage of spawning (stage 1, Plate 4E), resorption of unspawned oocytes progresses with the development of the next generation of oocytes. However, it is not clear how the old follicles in redeveloping stages redevelop and nurture the new sets of oogonia, and a similar uncertainty exists concerning the mechanism of resorption of unspawned oocytes.

Follicle development in the male reproductive system exhibits a similar peripheral arrangement to that of the females. Male individuals appeared to have a faster rate of gametogenic activity than females. Mature sperm are arranged with their acrosomes in a centripetal position and their tails occupying the central position of the lumen (Plate 5B). In this way, the classical conformation is established with the earliest stages lying near to the follicle wall (Plates 5C, F). Even in the most mature males some early spermatogenic stages are always found lining the follicle wall as a narrow band; these also persist after spawning and it seems probable that they act as a reservoir of germ cells for follicle redevelopment. Although very occasionally, male follicle development may be impaired by an unidentified parasite as shown in Plate 5H.

Some hermaphrodite individuals were encountered for *A. granosa* and *A. antiquata* in the present study. All had male and female tissue simultaneously in the same follicle rather than a separate testis and ovary. Plate 6 illustrates that hermaphrodites of both *A. granosa* or *A. antiquata*, discharged sperm whilst starting to develop oogonia. Quite often this commencement is not apparent, as only one or two oogonia slightly bigger than the spermatogonia and less intensely stained appeared marginally in the follicle wall. At this stage, the animals seemed not to produce a new layer of spermatogonia, but continued with the ripening process of spermatozoa. By

the time the spermatozoa had been almost totally discharged, the oogonia reached stage two developing. This is the last stage evident from the sections in this study. Perhaps they then continued the progress as pure female individuals. No evidence is available for the converse, i.e. females becoming male.

In this study, the smallest size at which reproductive tissue was observed histologically in *A. granosa* was when the animal had attained 15.6-15.7 mm shell length. Differentiated specimens less than 20mm long are mostly males. For *A. antiquata,* sections of 18.4-19.8mm animals revealed that they had not yet become sexually active. Some juveniles measuring 20.7-22.1mm showed mature gonads. The smallest females with ripe ovaries were found at 23-25mm shell length. In the sexually-undifferentiated clams of both species, the muscular wall of the visceral mass is separated from the gut and the digestive glands by only a thin layer of connective tissue. At this stage, the digestive glands occupied 1/3 or more of the body cavity in cross section.

3.3.2. Sexuality and Sex Ratio

Both species of *A. granosa* and *A. antiquata* are dioecious. Hermaphrodites occurred in only 1.43 and 1.45% (6 out of 420 and 4 out of 276 individuals) for the populations of *A. granosa* in Wedung and Tapak respectively. For *A. antiquata* in Bandengan the figure is somewhat lower, i.e. 0.84% or 3 out of 356 individuals

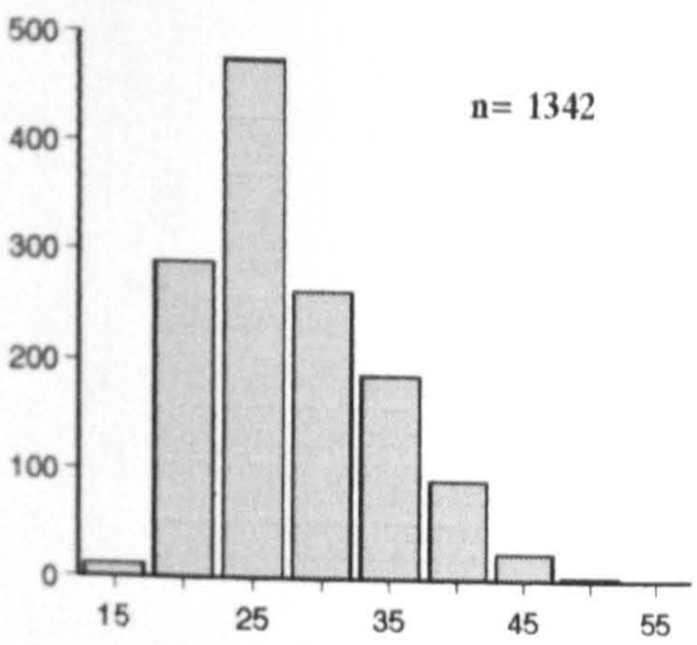
Apparently both species have a protandric type of development in which a primary male phase precedes the adult stages until both sexes are approximately equally represented, after which sex reversal takes place. Figure 13 supports this suggestion at the population level. In Wedung and Bandengan, the overall ratio of males to females is 1.49:1 (χ^2= 120.08;

Figure 13: Size frequency distribution and sex ratios of male and female *A. granosa* and *A. antiquata* collected during a 24 month period from the populations in:

A. Wedung, *A. granosa*
B. Tapak, *A. granosa* and
C. Bandengan, *A. antiquata*

Only those individuals whose sexes can be confidently determined from the external appearance of their gonads were used to construct the histograms. For all populations the sex ratios depart from a ratio of 1:1 with 99% level of confidence.

A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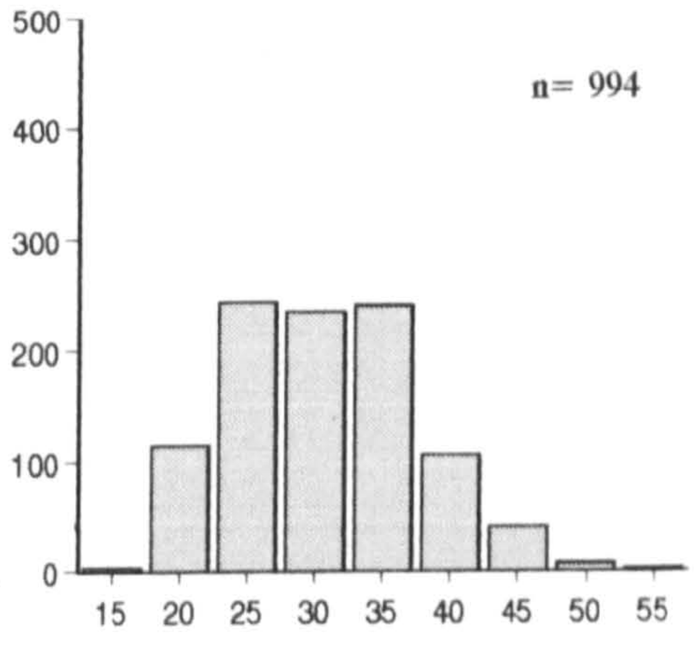
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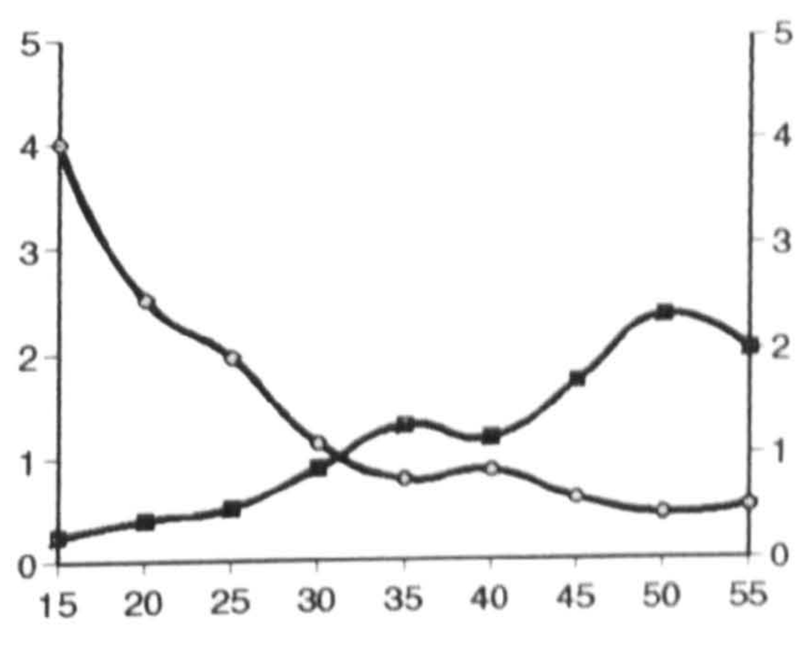

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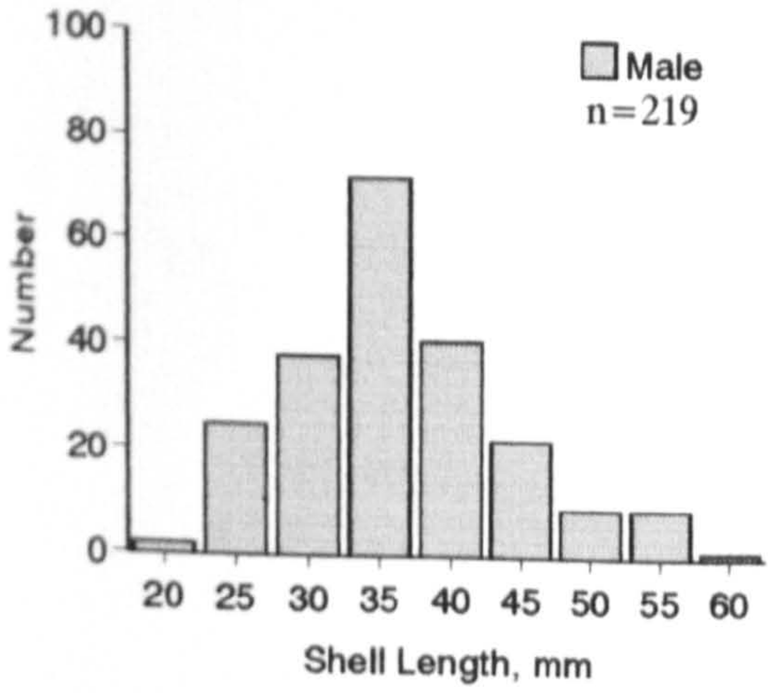
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Number
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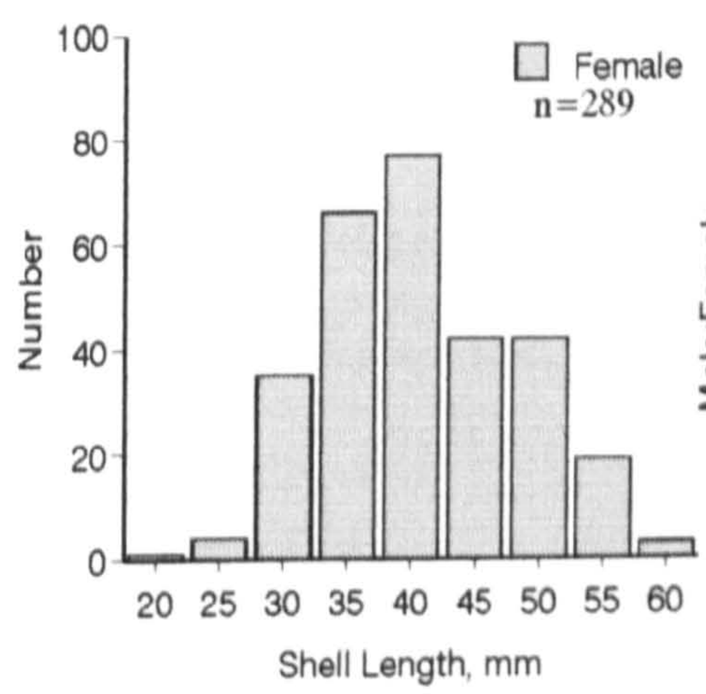
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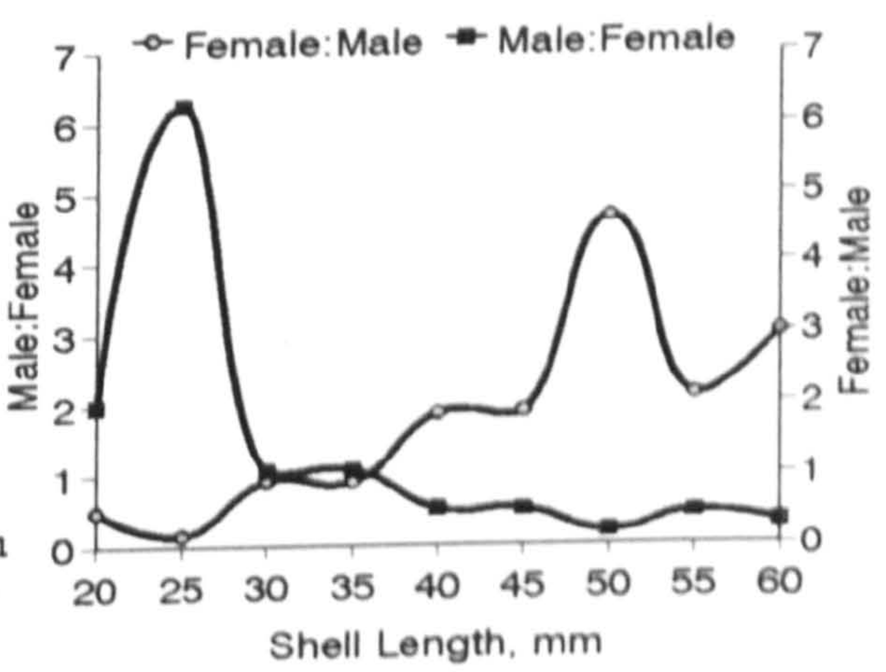
Number
Shell Length, mm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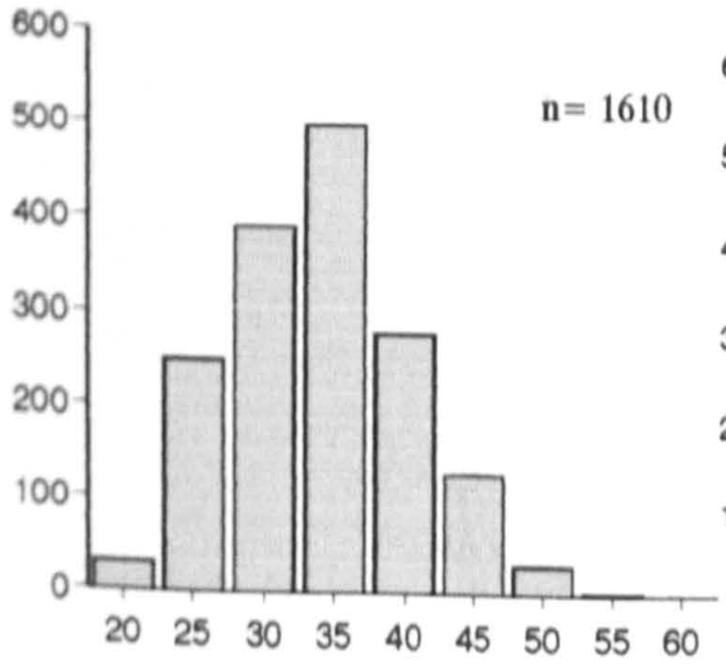
Male:Female
Male:Female
Female:Male
Shell Length, mm

C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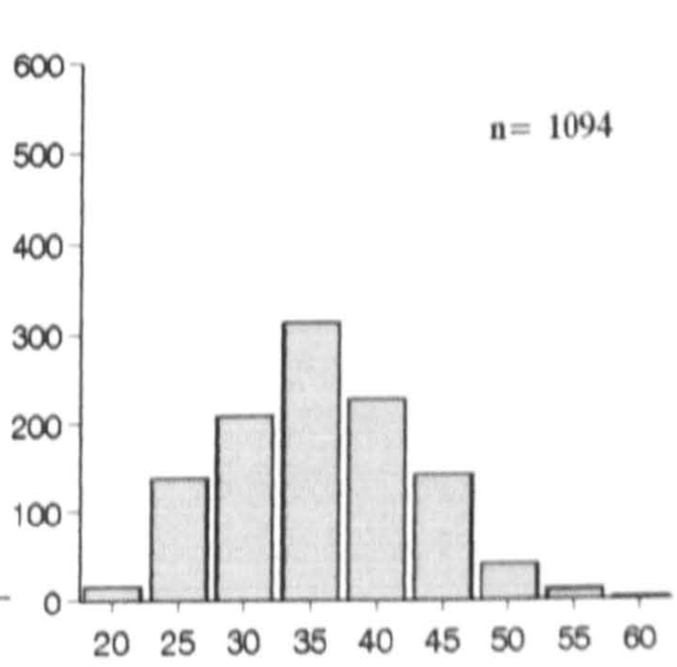
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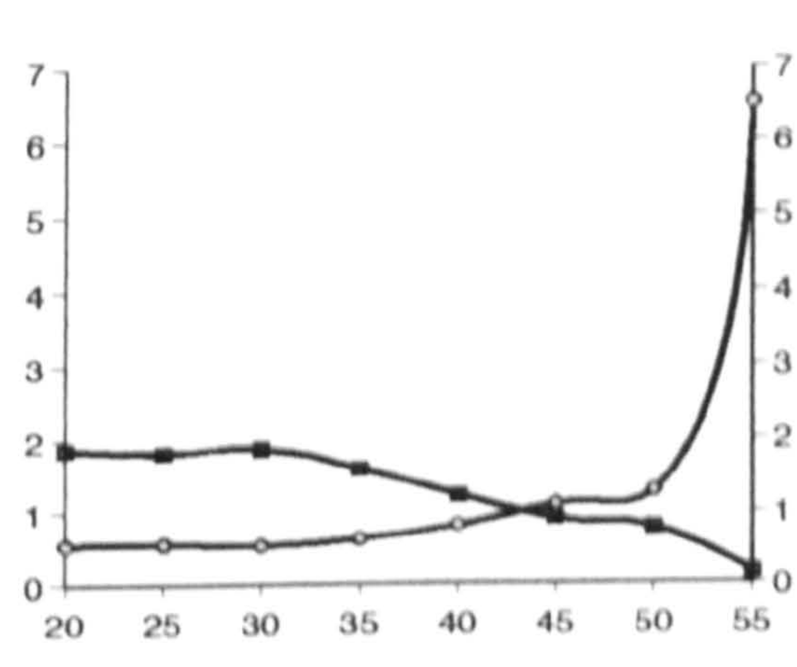


Plate 4: Photomicrographs of sectioned female gonads of *A. granosa* at various stages of development

A. Developing female stage 1, showing a number of early stage oogonia with some elongated oogonia, x250.

B. Developing female stage 2, stalked oocytes attached to the germinal epithelium of the follicle, x400.

C. Ripe female stage 3, mature oocytes at maximum density. Note the somewhat hexagonal shape as they were densely packed, x250.

D. Spawning female stage 2, empty spaces left by the discharged oocytes appear in the centre of the lumen, as a result of the reduced pressure the oocytes appear rounded in shape, x250.

E. Spawning female stage 1, empty lumen with thin follicle wall and some residual rounded oocytes, x250.

F. Spent stage, completely empty follicle. Sex is undetermined, x250.

G. Redeveloping female stage 1, a number of early stage oogonia and some residual oocytes in large, elongated and relatively empty follicles, x100.

H. Redeveloping female stage 2, follicles are approximately full of developing oogonia and mature oocytes, x100.

A
B
C
D
E
F
G
H

Plate 5: Photomicrographs of sectioned male gonads of *A. granosa* at various stages of development

A. Ripe male stage 3 at low magnification, x100.

B. Ripe male stage 3 at higher magnification, sperm are arranged with their acrosomes in a centripetal position and their tails occupying the central position of the lumen, x250.

C. Spawning male stage 2, with layers of ready-to-develop spermatogonia lining the follicle wall, x250.

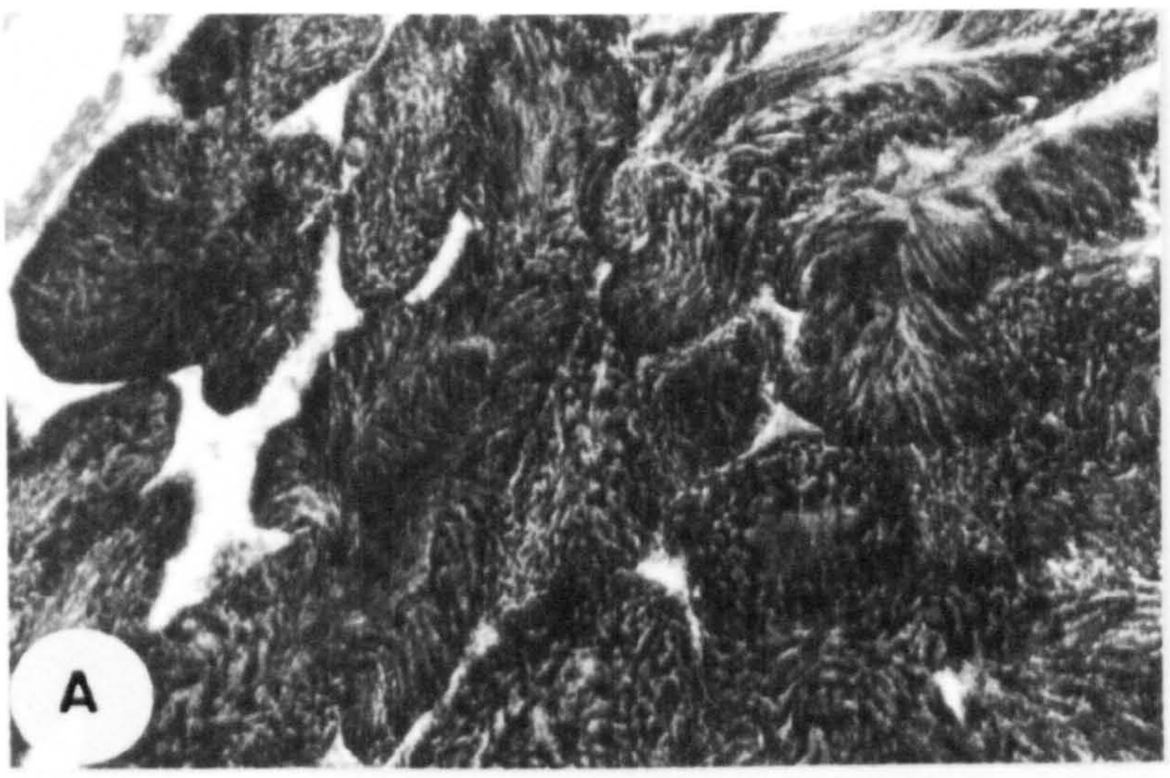
D. Spawning male stage 1, some lumen contain residual sperm, the other are completely spent, x250.

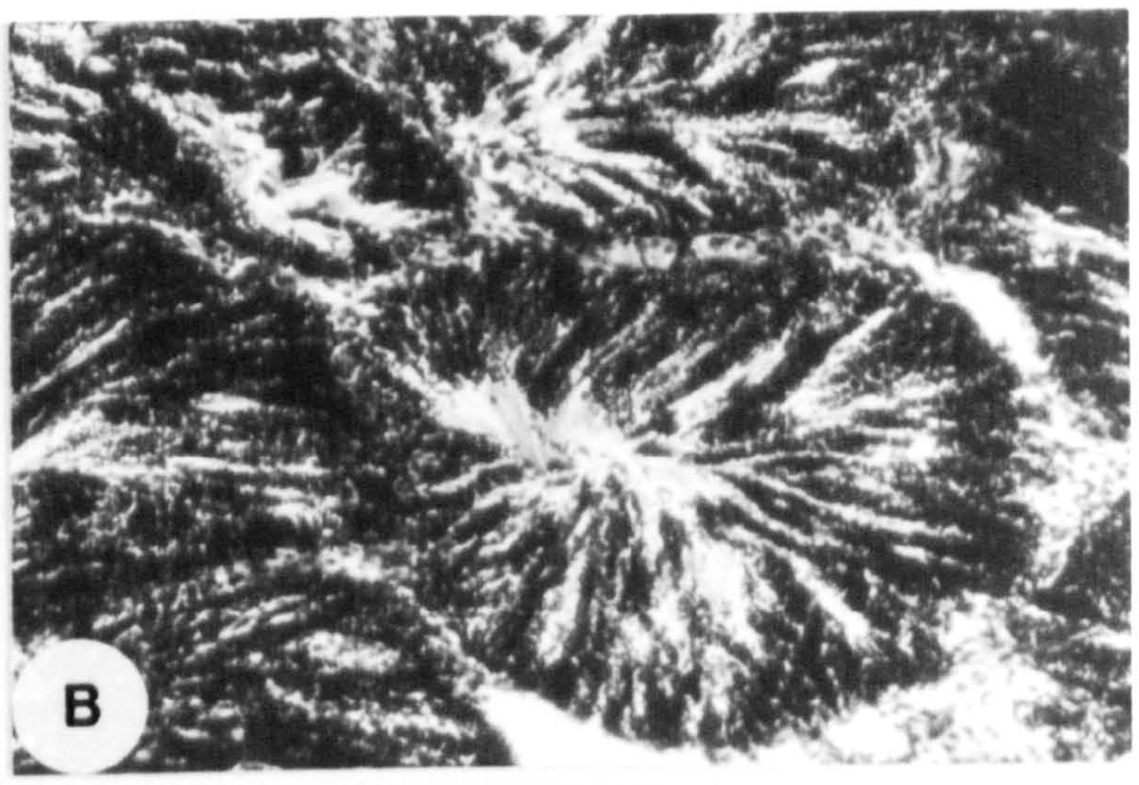
E. Empty follicle at the spent stage, x100.

F. Redeveloping male stage 1, a layer of undifferentiated early stage spermatogonia line the empty follicle, x250.

G. Redeveloping male stage 2, a broad layer of developing spermatogonia. The spermatids can be differentiated under high magnification, x250.

H. Male follicle impaired by unknown parasites, x250.


A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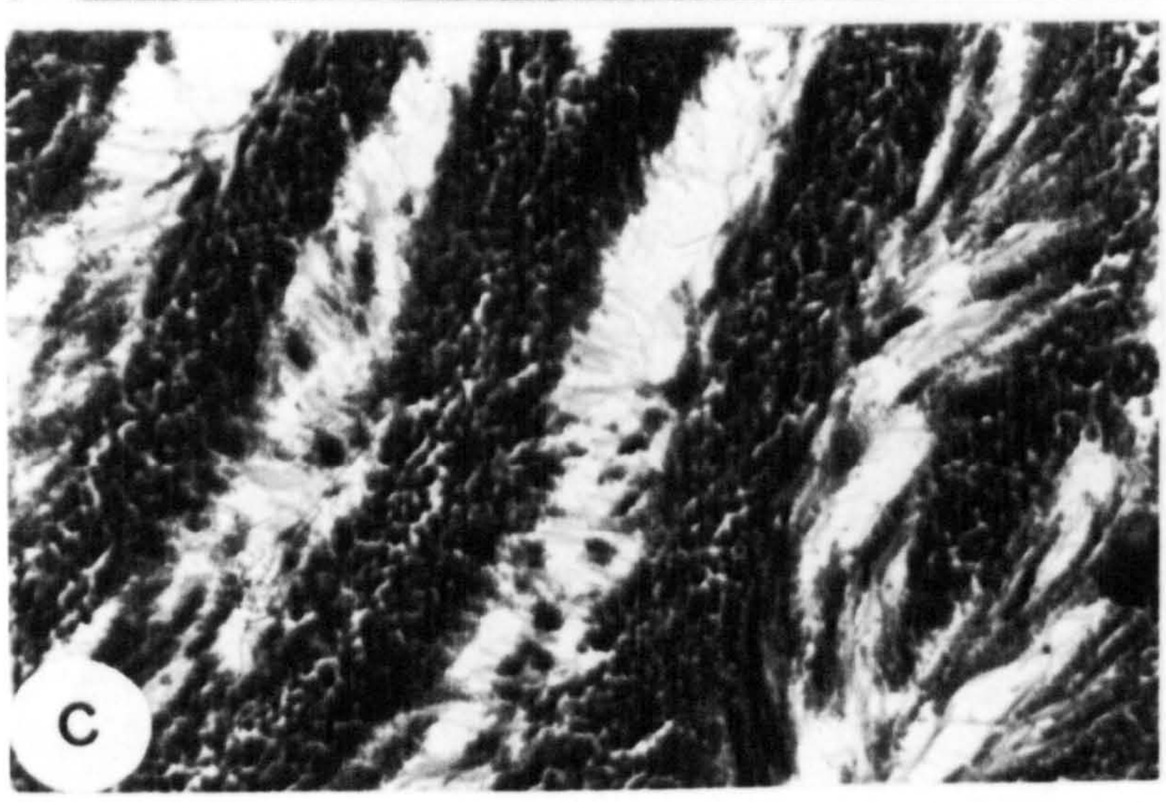
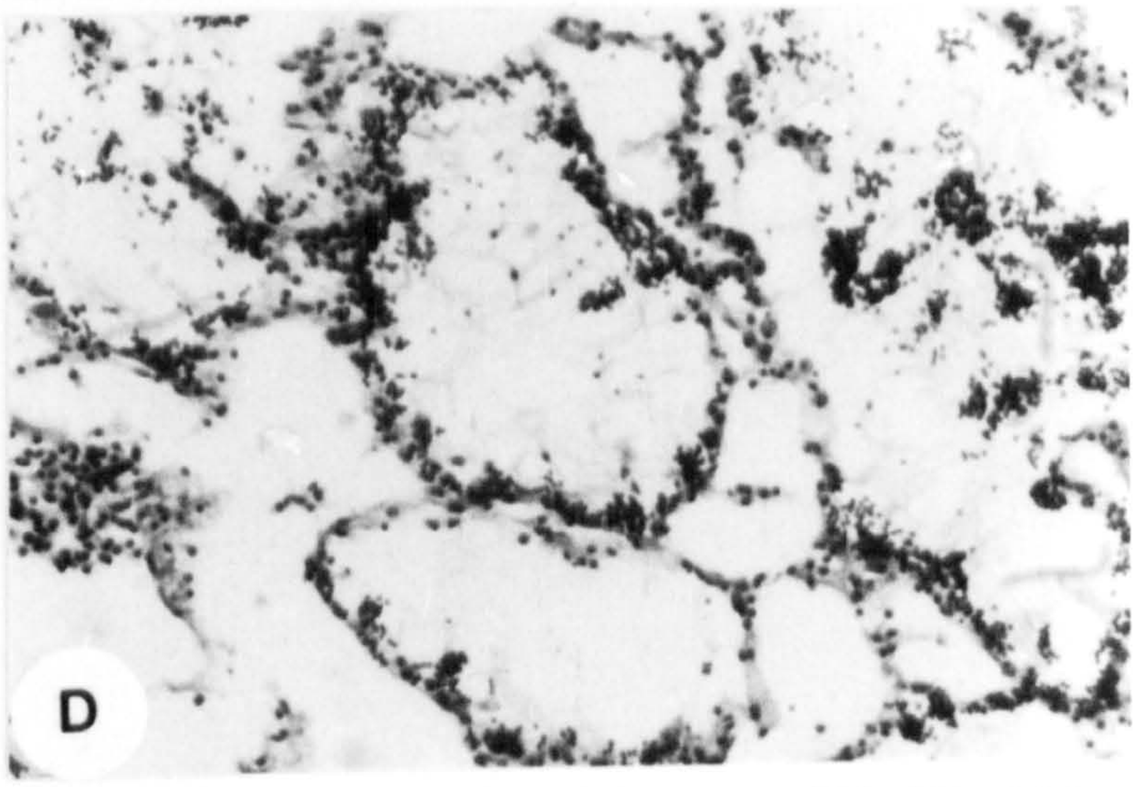
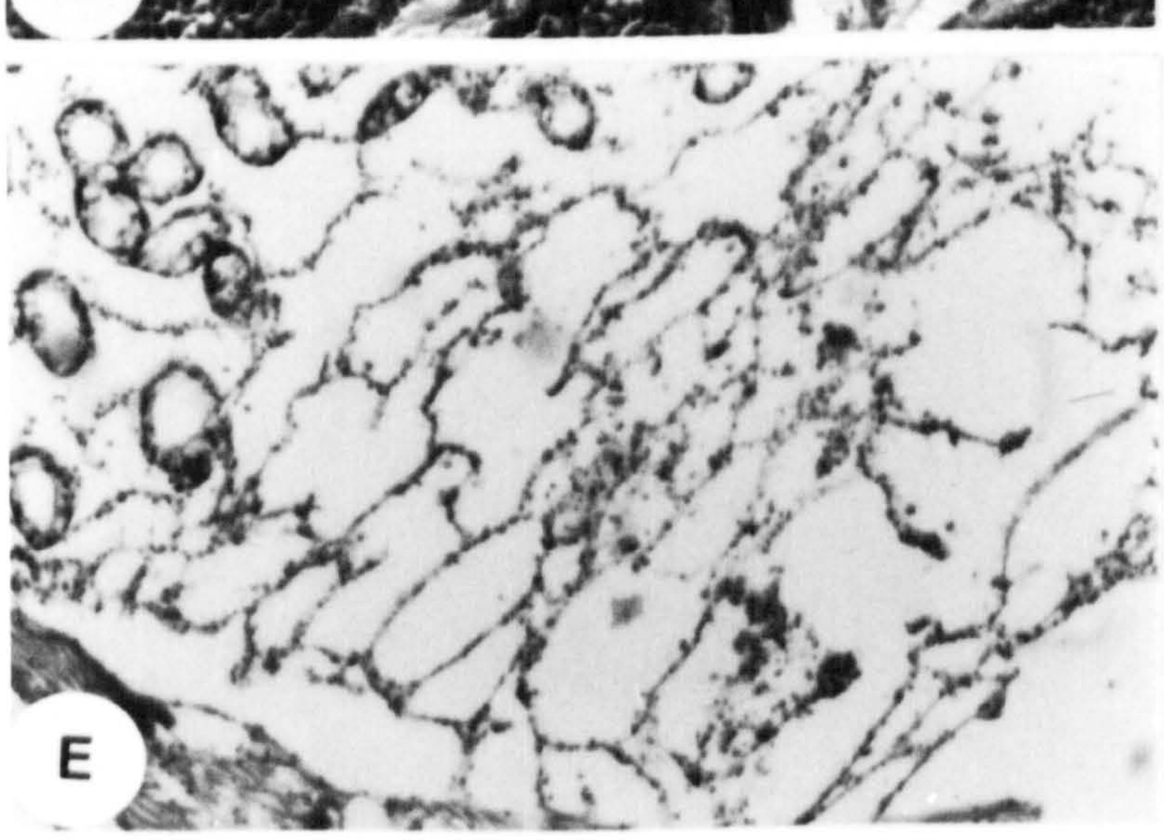
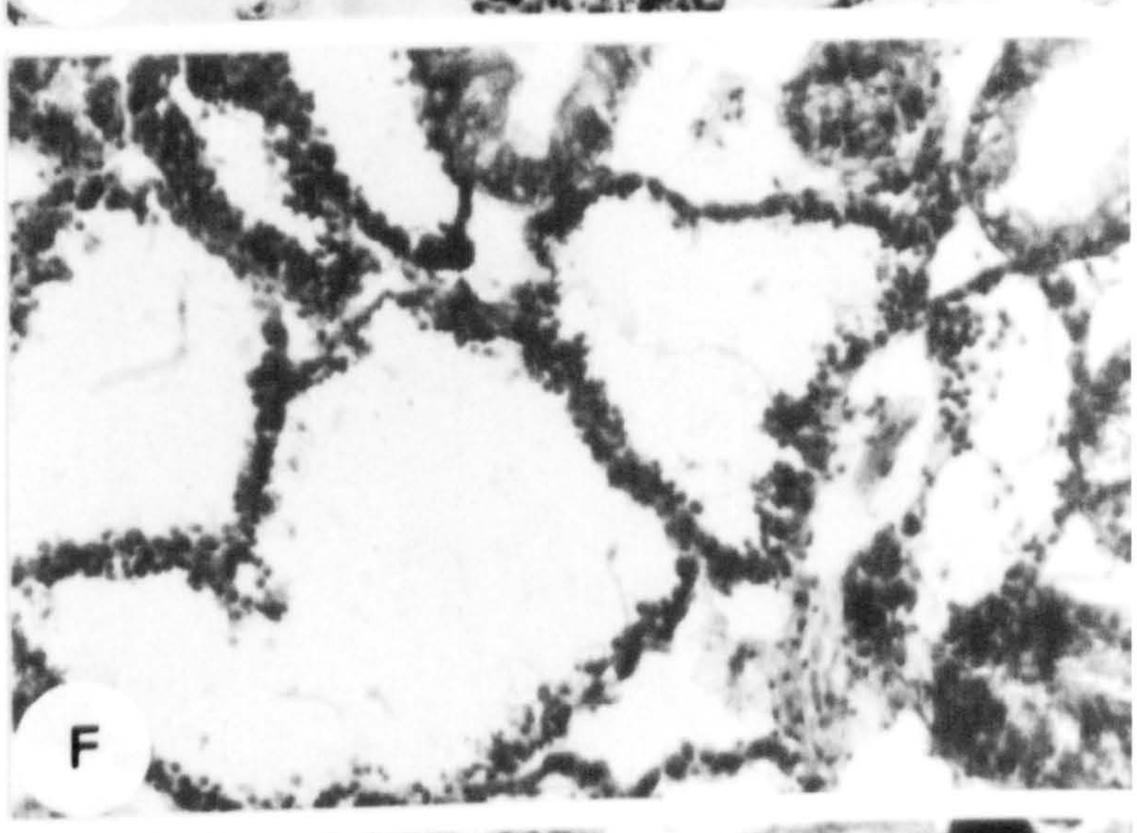
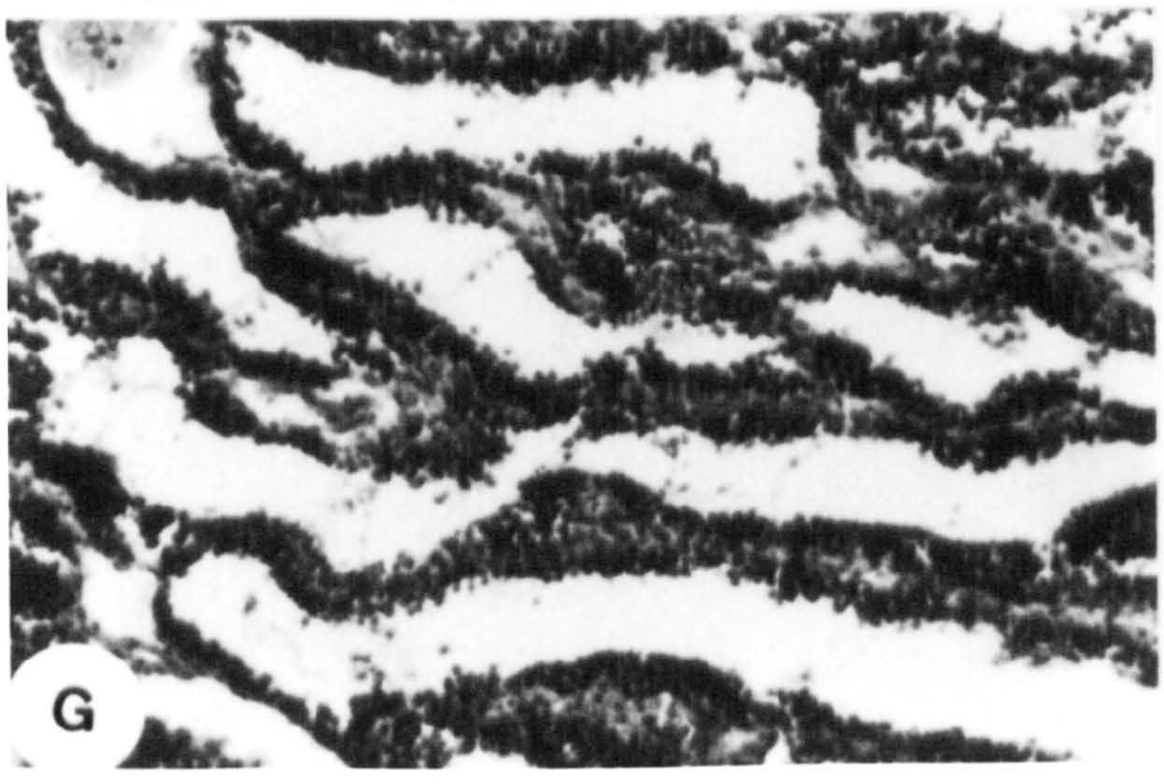

C


D


E


F


G

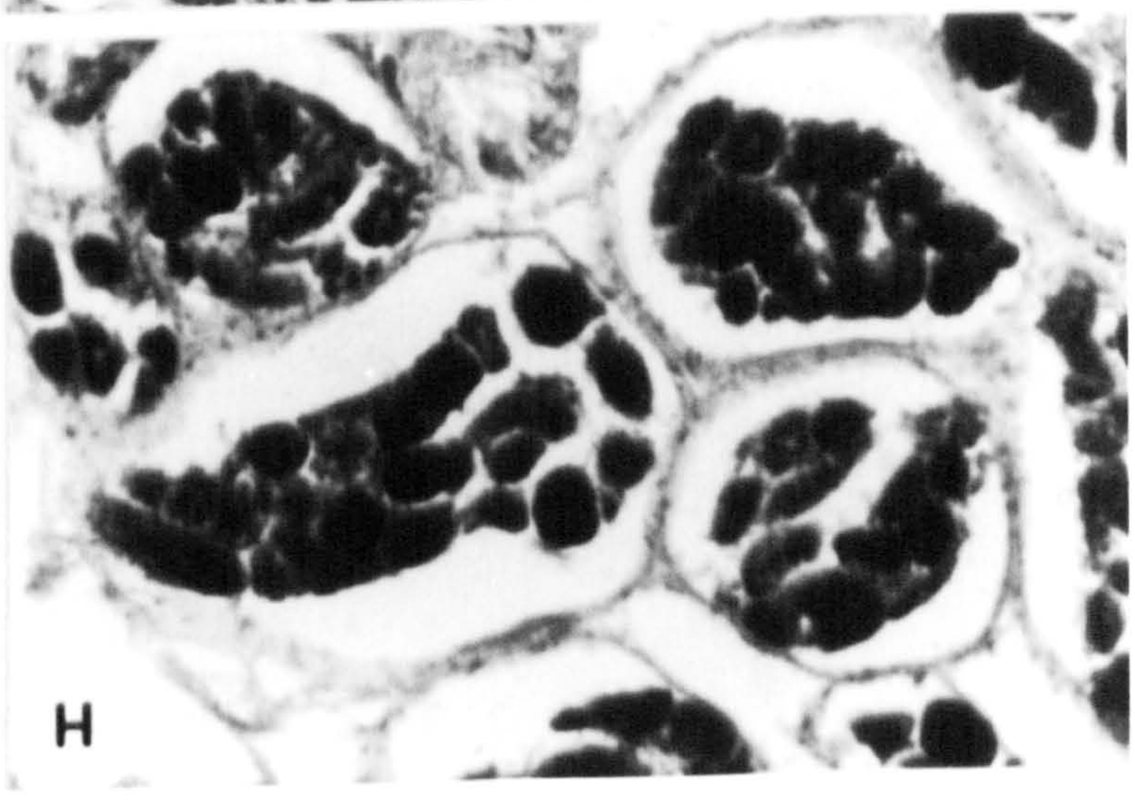
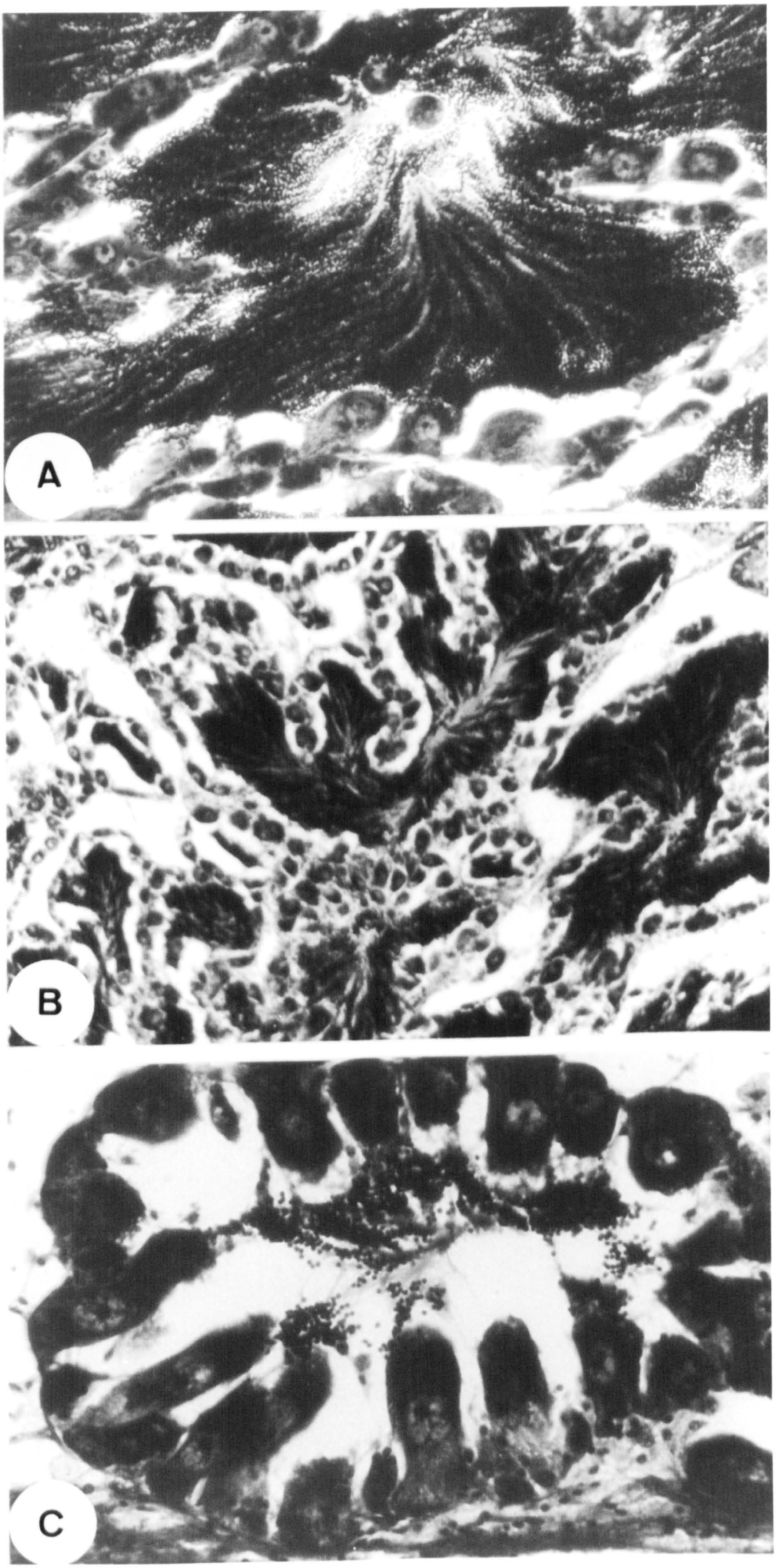

H

Plate 6: Photomicrographs of sectioned gonads of hermaphodites *A. granosa* and *A. antiquata*

A. Hermaphrodite individual discharging its sperm whilst starting to develop the female reproductive tissue, x250.

B. The appearance of hermaphrodites with a layer of fusiform oogonia flattened to the follicle wall, x100.

C. Further stage in sex changing in hermaphrodites, oogonia becoming elongated whilst a few residual sperm were still in the centre of the follicle, x400.


A
B
C

P<0.001, df= 7, n= 2342) and 1.47:1 (χ^2= 51.13; P<0.001, df= 6, n= 2074) respectively whereas in Tapak it is 0.76:1 but in favour of the females (χ^2=49.90; P<0.001,df= 6, n= 508). So, in all populations there was a significant departure from a 1:1 ratio. Only when *A. granosa* attained a size of 30-35 mm in shell length did the sex ratio approach 1:1; for *A. antiquata* this occurred when the animals were 40-45 mm long (Fig. 13).

The histograms in Figure 13A show that in the Wedung population, the mode for male individuals is 25 mm, thereafter the number of male blood clams decreased rapidly at 30 mm onwards, whereas the number of females remained constant from 25mm until they are at about 40 mm long (Fig. 13A). Similarly in Tapak, the mode for males is at 35 mm, and 40 mm for females (Fig. 13B). In Bandengan, although the mode for male and female *A. antiquata* appeared to be the same at 35 mm, but only when they attained a size of 40-45mm did the sex ratio approach 1:1 (Fig.13C). It was also noteworthy that in Wedung and Bandengan, the number of male blood clams is always higher than that of the females but this was not the case for the Tapak population.

3.3.3. Seasonal changes in reproductive condition

Figures 14 to 16 illustrate A) records of the annual gonad stage in both males and females from subsamples in the monthly samples, B) the seasonal changes in size distribution of oocytes, and C) density of oocytes, as well as D) the mean male and female gonad indices for both species in the three study sites. The average size of oocytes throughout the study period was divided into three categories, i.e. small (5-20μm), medium (25-40μm) and large (45-65μm; Table 7). In general, the male gonad index is more constant and relatively higher than that of the females, particularly for *A. antiquata*

which exhibit a more seasonal pattern than *A. granosa*. Also, it can be seen from Figs. 14D-15D that the overall mean male gonad index in Tapak is lower than in Wedung.

Irrespective of the time of collection and size of the individual clams, the average density of oocytes in *A. antiquata* is much lower than that of *A. granosa*, suggesting a lower fecundity (Table 7). On the basis of size composition and oocyte densities amongst the populations of *A. granosa*, it seems that the Tapak population maintained more continuous reproductive activity by having a relatively higher percentage of small oocytes in reserve, albeit that the average density of oocytes is lower than amongst female specimens in Wedung (Table 7). However, apart from the distinct spawning seasons in October-December 1991 and June-August 1993, the narrow range of confidence interval for the oocyte densities of *A. granosa* from Wedung suggested a relatively uniform reproductive condition amongst member of the population. This means that they were approximately in the same stages of reproduction at any given time (Fig. 14C).

A wider range of confidence interval for oocyte densities was noted for Tapak and particularly for the Bandengan populations (Figs. 15C and 16C), suggesting more variability amongst female individuals with respect to their reproductive condition. In their major spawning period for example, i.e. June and August 1993 for the Wedung population or July-August 1993 for the Tapak population, more than 50% of the population was still in the various stages of development. This contrasts with *A. antiquata* when at certain times of the year about 80% of the population was fully spent (November 1991, October-November 1992), whilst the rest was spawning (compare Figs. 14 and 15A-C to Figs. 16A-C). The fully spent stage in Tapak on May 1992 however, is based on only two individuals, so more information is required

Table 7: The average density and the percentage of the overall mean oocytes size for *A. granosa* in Wedung and Tapak and *A. antiquata* in Bandengan throughout the study period (± Standard Deviation).

Parameters	Populations		
	A. granosa		*A. antiquata*
	Wedung	Tapak	Bandengan
Average Density, mm^{-2}	471.24 ±75.48	440.08 ± 154.97	272.29 ± 176.39
Size range of oocytes			
5-20μm	25.47 ± 7.46	39.02 ± 14.94	24.98 ± 16.20
25-40μm	57.09 ± 9.31	50.80 ± 11.82	62.95 ± 18.58
45-65μm	17.44 ± 7.11	10.18 ± 8.35	7.72 ± 7.18

Figure 14: Reproductive seasonality of *A. granosa* from Wedung.

A. Records of the four main gonad stages of male and female clams categorised from histological sections. Stages shown at the top of the figure.

B. Size composition of oocytes, categorised as small (5-20μ), medium (25-40μ) and large (>45μ). The monthly size range of oocytes on which this figure are based is presented in Appendix 3.

C. Average density of oocytes calculated from sections of individual females in the monthly samples. Bars represent the range of the 95% confidence interval.

D. The seasonal variation in the average gonad indices for male and female *A. granosa*.

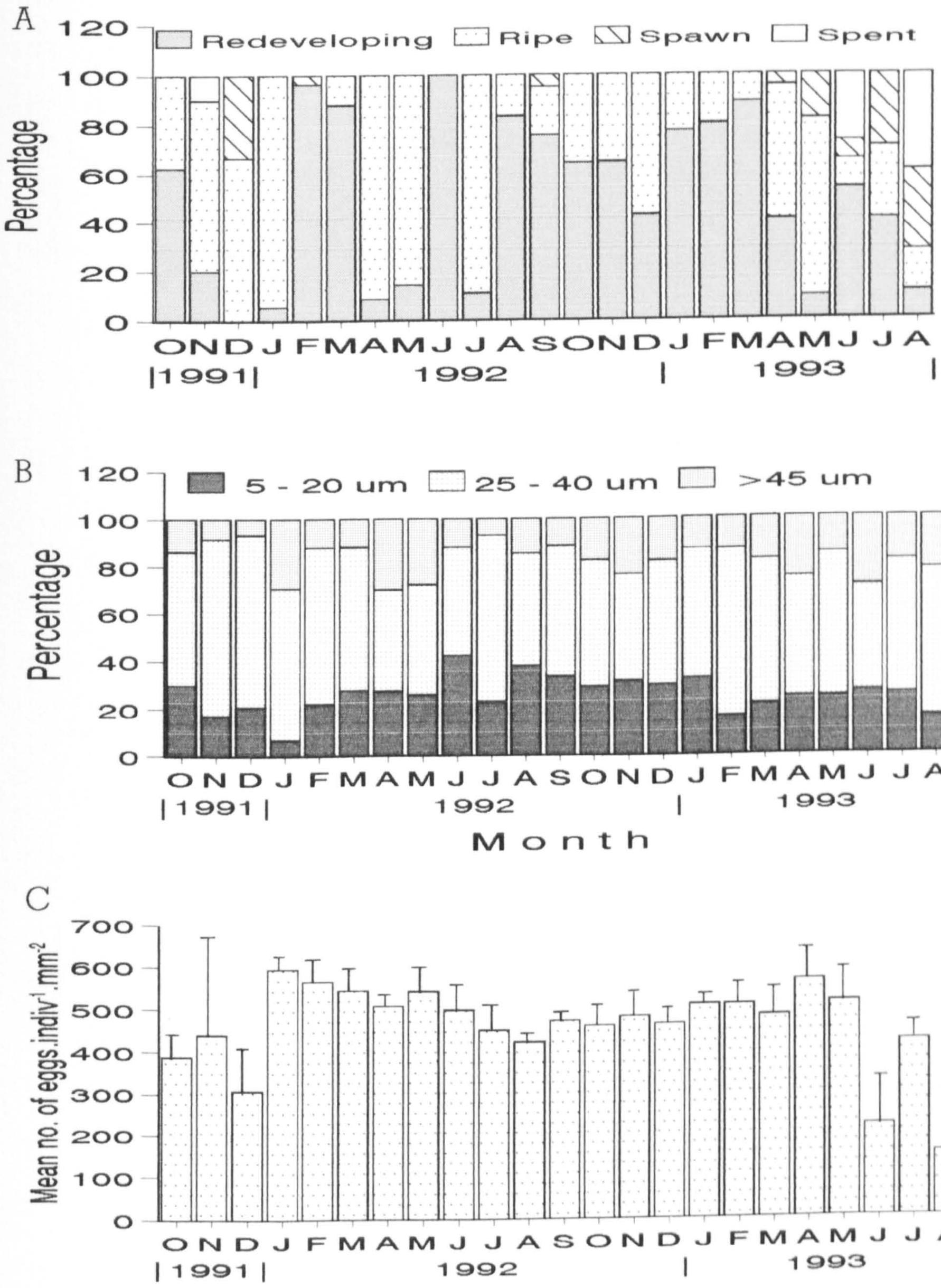

A
Redeveloping
Ripe
Spawn
Spent
Percentage
120
100
80
60
40
20
0
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
5 - 20 um
25 - 40 um
>45 um
Percentage
Month
C
Mean no. of eggs.indiv-1.mm-2
700
600
500
400
300
200
100
0
D
Mean Gonad Index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Female
Male

Figure 15: Reproductive seasonality of *A. granosa* from Tapak.

A. Records of the four main gonad stages of male and female clams categorised from histological sections. In April and May 1993 samples were not available. Stages shown at the top of the figure.

B. Size composition of oocytes, categorised as small (5-20μ), medium (25-40μ) and large (>45μ). The monthly size range of oocytes on which this figure are based is presented in Appendix 3. In May 1992 only two female specimens were collected and both were in the completely spent stage.

C. Average density of oocytes calculated from sections of individual females in the monthly samples. Bars represent the range of the 95% confidence interval.

D. The seasonal variation in the average gonad indices for male and female *A. granosa*.

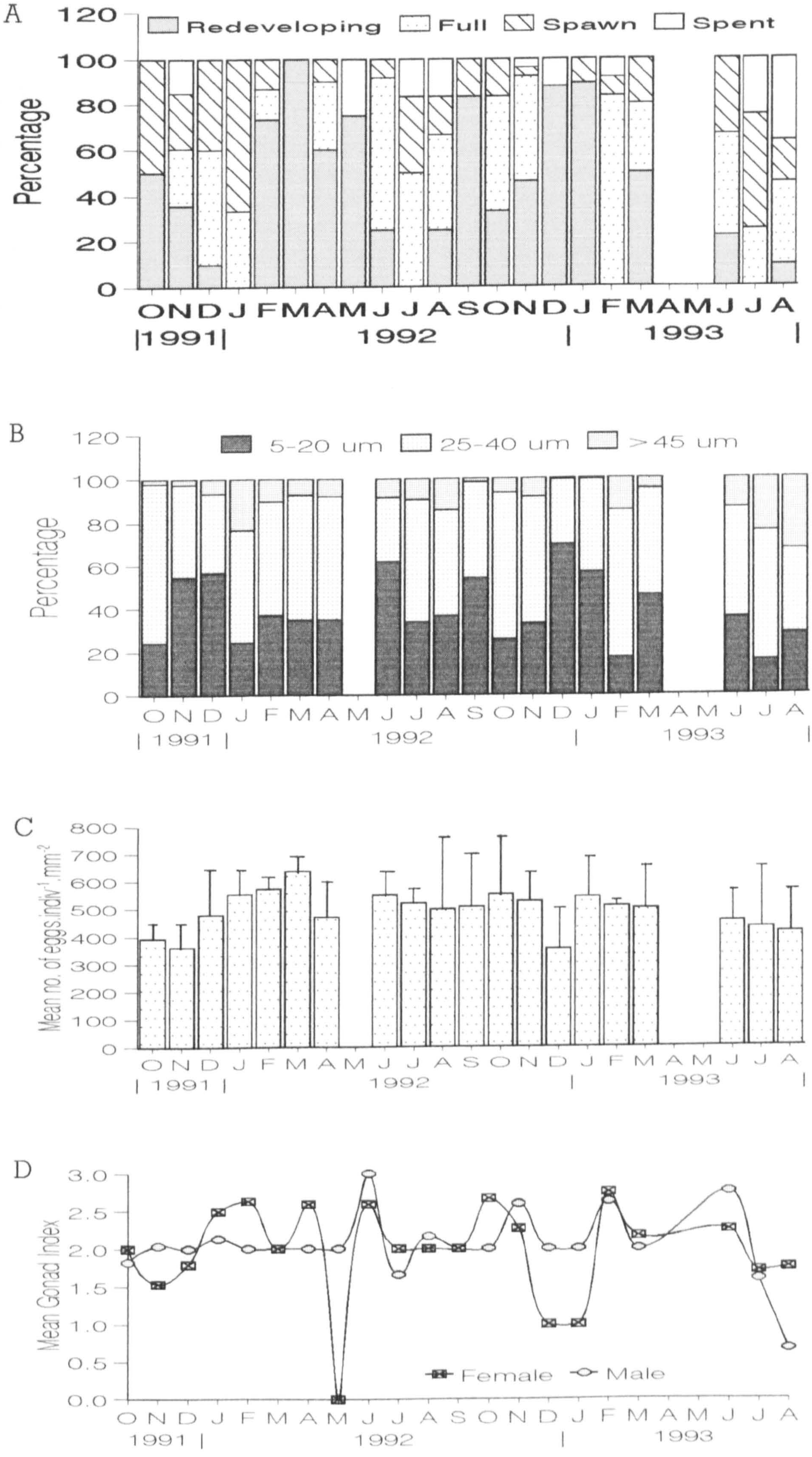

A
120
100
80
60
40
20
0
Percentage
Redeveloping
Full
Spawn
Spent
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
120
100
80
60
40
20
0
Percentage
5-20 um
25-40 um
>45 um
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
C
800
700
600
500
400
300
200
100
0
Mean no. of eggs.indiv-1.mm-2
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
D
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Mean Gonad Index
Female
Male
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

Figure 16: Reproductive seasonality of *A. antiquata* from Bandengan.

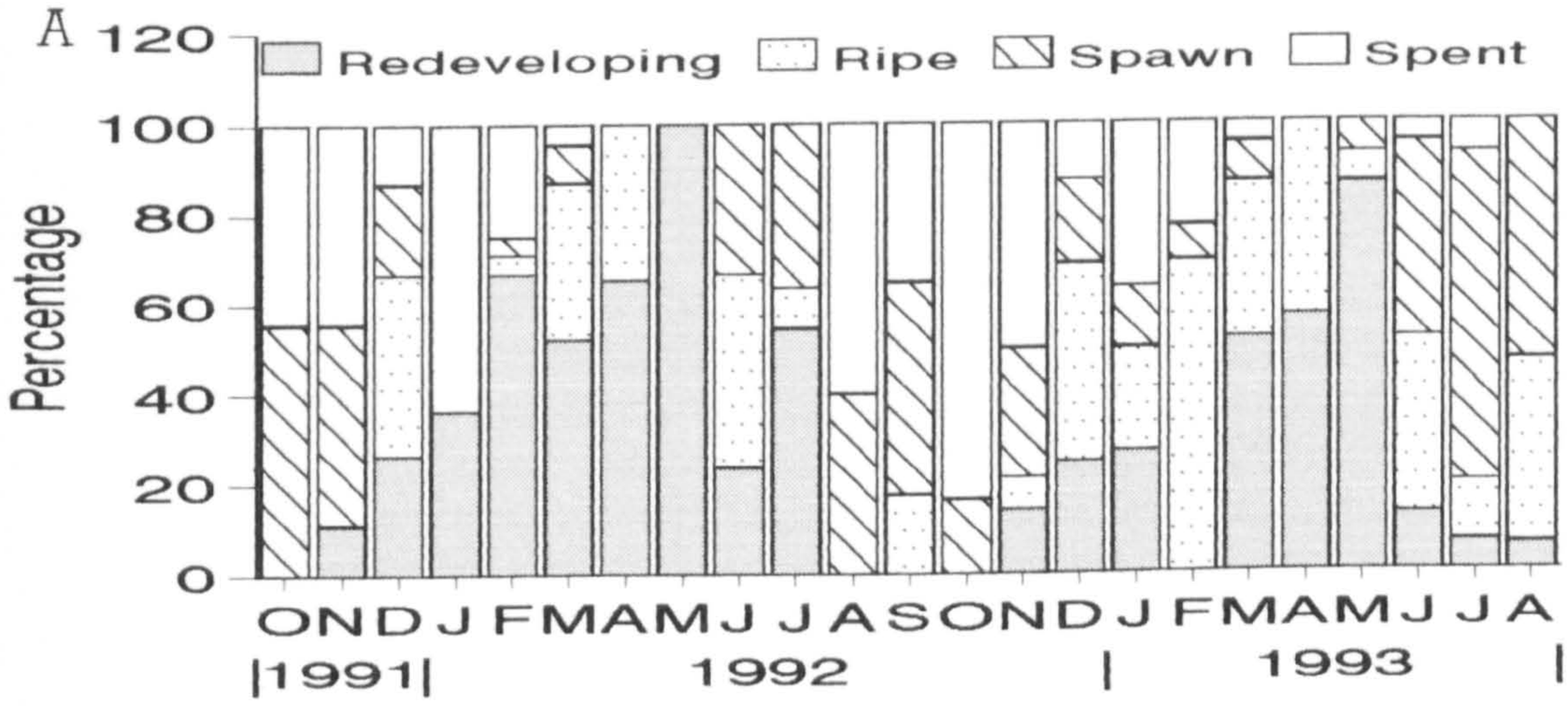
A. Records of the four main gonad stages of male and female clams categorised from histological sections. Stages shown at the top of the figure.

B. Size composition of oocytes, categorised as small (5-20μ), medium (25-40μ) and large (>45μ). The monthly size range of oocytes on which this figure are based is presented in Appendix 3. In October 1992 female specimens were all in the complete spent stage.

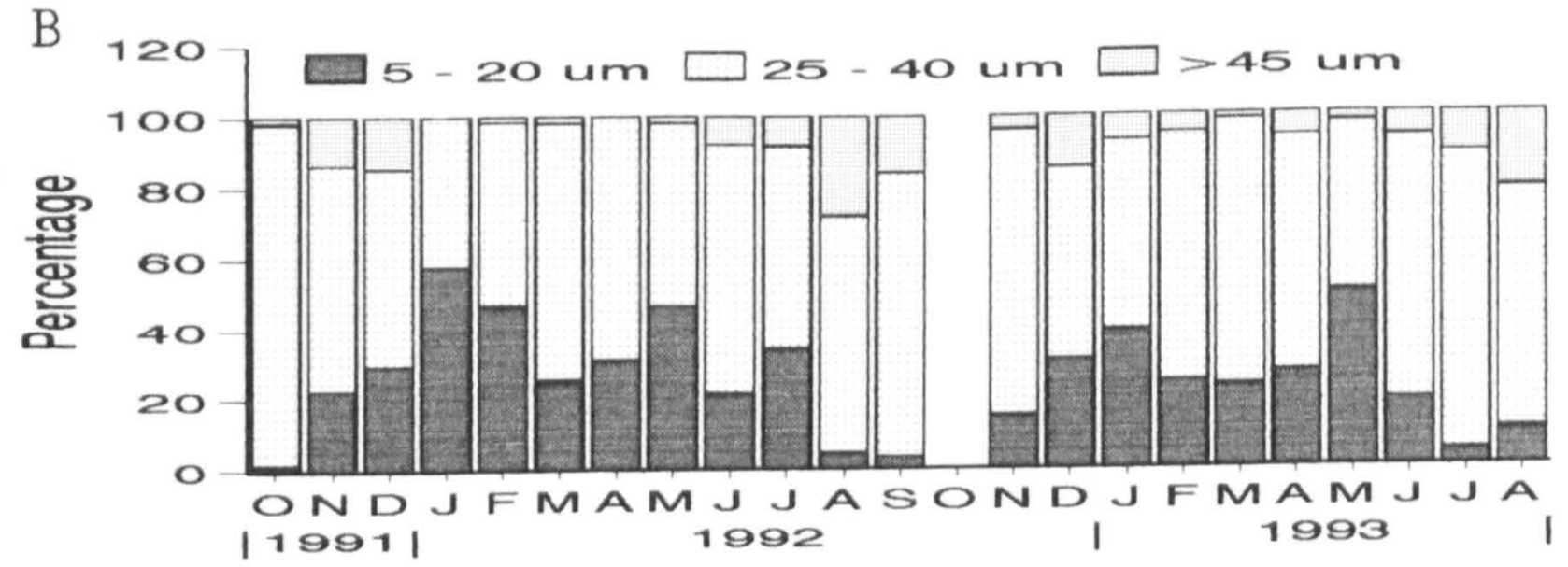
C. Average density of oocytes calculated from sections of individual females in the monthly samples. Bars represent the range of the confidence interval.

D. The seasonal variation in the average gonad indices for male and female *A. antiquata*.

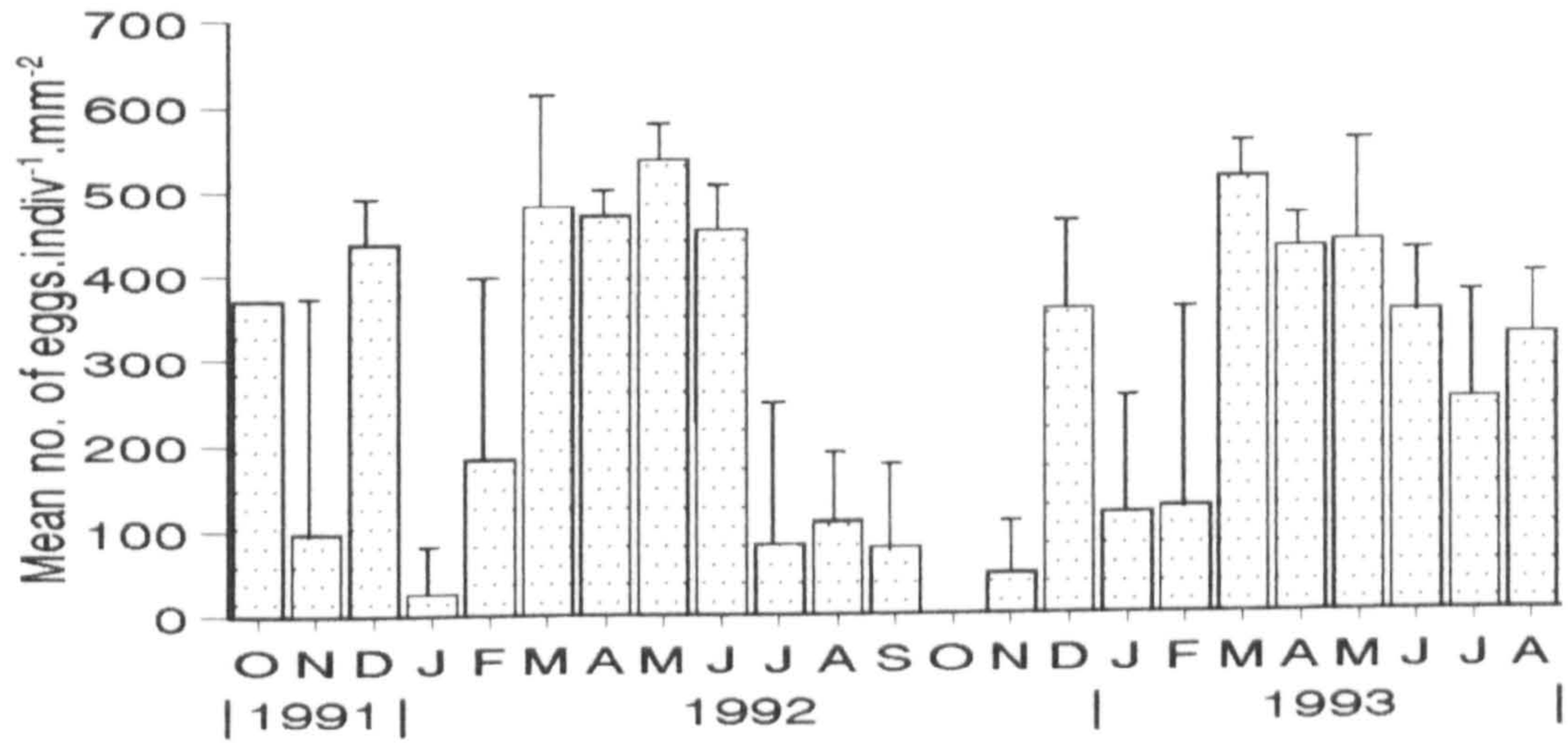
A


Redeveloping
Ripe
Spawn
Spent
Percentage
120
100
80
60
40
20
0
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

B


5 - 20 um
25 - 40 um
>45 um
Percentage
120
100
80
60
40
20
0
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

C


Mean no. of eggs.indiv-1.mm-2
700
600
500
400
300
200
100
0
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

D

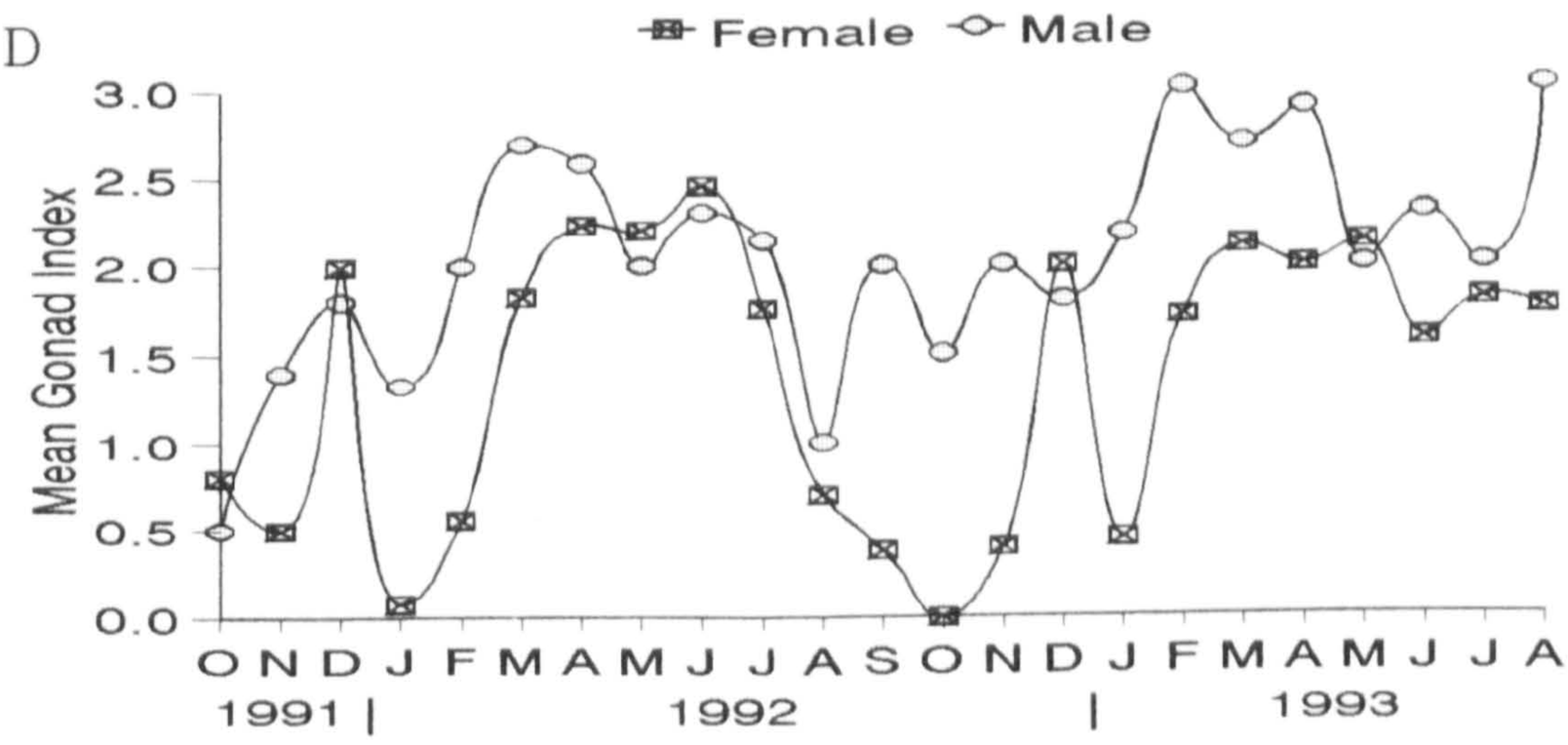

Female
Male
Mean Gonad Index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

Figure 17: Length frequency histograms for monthly population samples of *A. granosa* from Wedung, showing the composition of male, female and undifferentiated individuals throughout the study period. Numbers represent total number of individuals in that particular month. Major recruitments occurred in August-September 1991, August-September 1992 and July-August 1993.

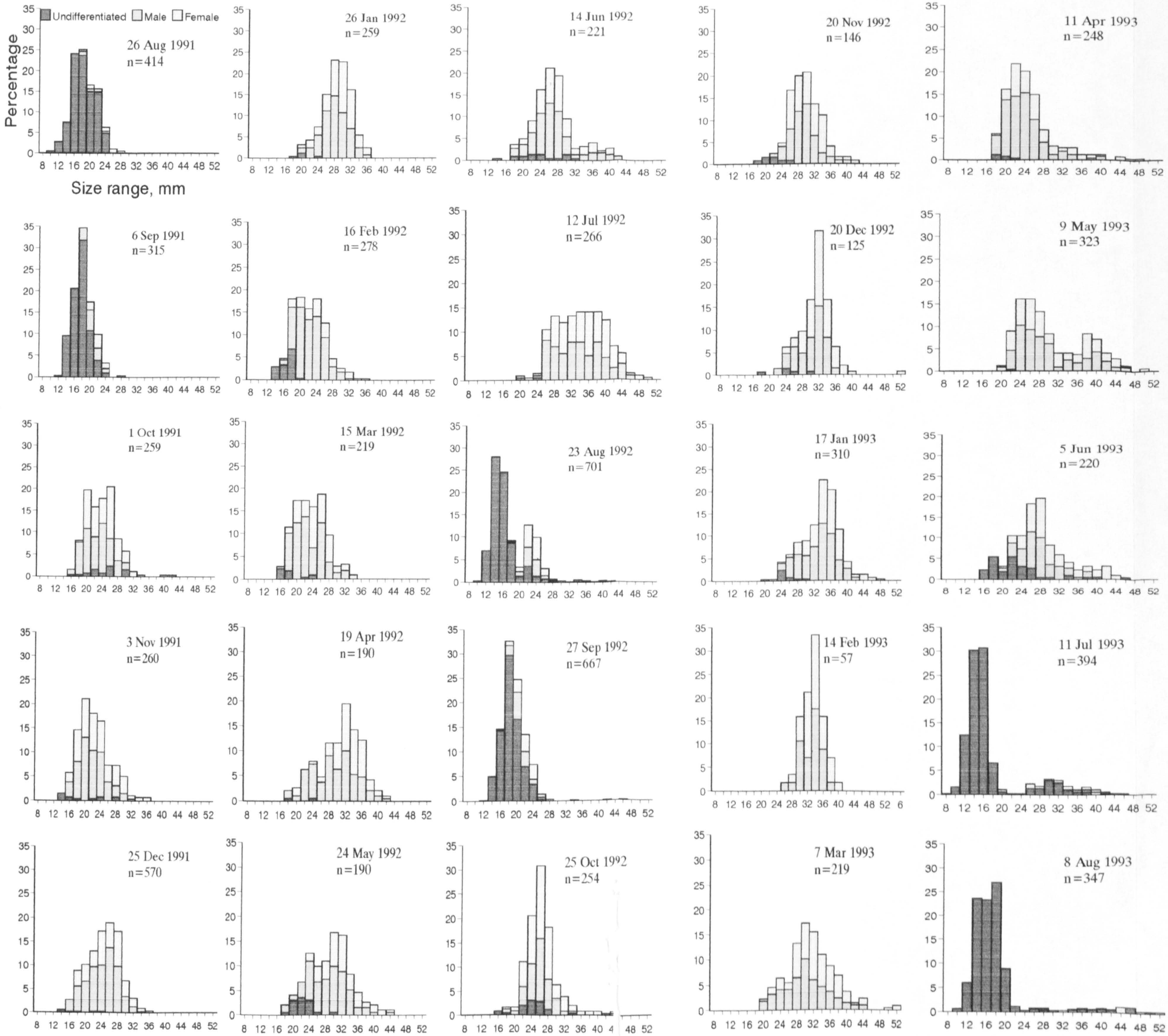

Undifferentiated
Male
Female
Percentage
Size range, mm
26 Aug 1991
n=414
26 Jan 1992
n=259
14 Jun 1992
n=221
20 Nov 1992
n=146
11 Apr 1993
n=248
6 Sep 1991
n=315
16 Feb 1992
n=278
12 Jul 1992
n=266
20 Dec 1992
n=125
9 May 1993
n=323
1 Oct 1991
n=259
15 Mar 1992
n=219
23 Aug 1992
n=701
17 Jan 1993
n=310
5 Jun 1993
n=220
3 Nov 1991
n=260
19 Apr 1992
n=190
27 Sep 1992
n=667
14 Feb 1993
n=57
11 Jul 1993
n=394
25 Dec 1991
n=570
24 May 1992
n=190
25 Oct 1992
n=254
7 Mar 1993
n=219
8 Aug 1993
n=347

Figure 18: Length frequency histograms for four-monthly population samples of *A. granosa* from Tapak, showing the composition of male, female and a few undifferentiated individuals.No distinct recruitment period was distinguished throughout the study period. Numbers represent total number of individuals within the four month collections. Samples were not available for April and May 1993.

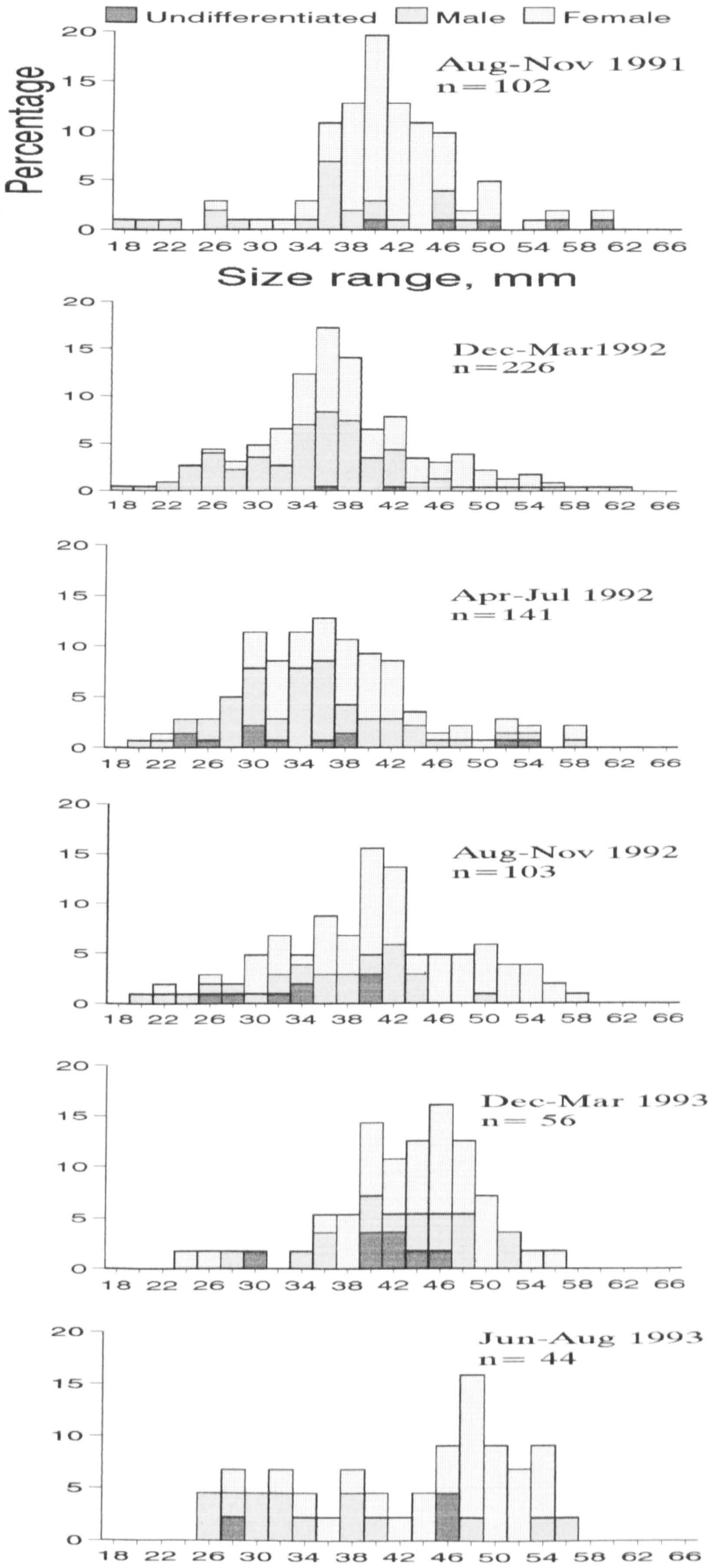
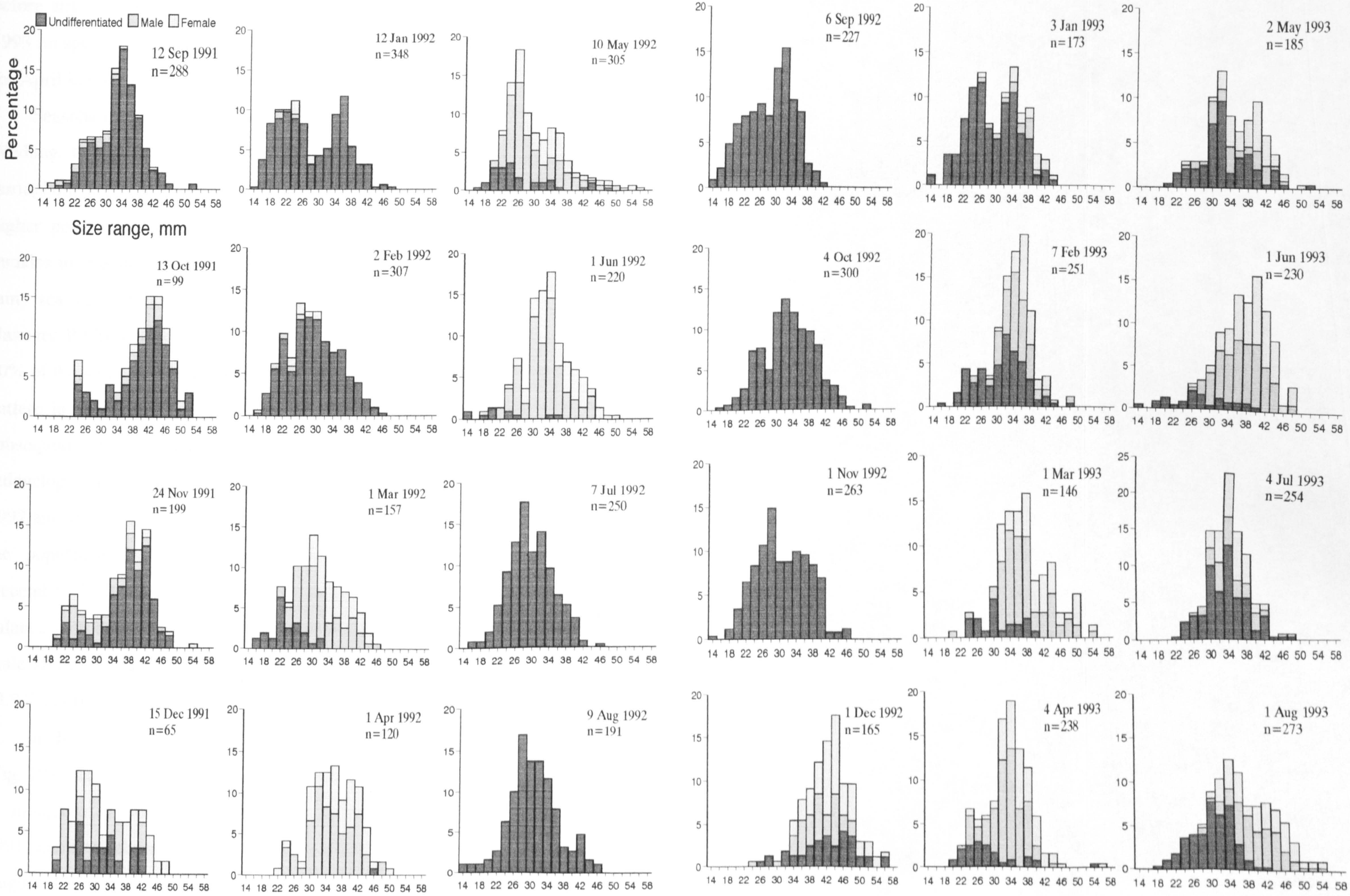

Undifferentiated
Male
Female
Percentage
Size range, mm
Aug-Nov 1991
n=102
Dec-Mar1992
n=226
Apr-Jul 1992
n=141
Aug-Nov 1992
n=103
Dec-Mar 1993
n= 56
Jun-Aug 1993
n= 44
0
5
10
15
20
18 22 26 30 34 38 42 46 50 54 58 62 66

Figure 19: Length frequency histograms for monthly population samples of *A. antiquata* from Bandengan, showing the composition of male, female and undifferentiated throughout the study period. Numbers represent total number of individuals in that particular month.


Undifferentiated
Male
Female
Percentage
Size range, mm
12 Sep 1991
n=288
12 Jan 1992
n=348
10 May 1992
n=305
6 Sep 1992
n=227
3 Jan 1993
n=173
2 May 1993
n=185
13 Oct 1991
n=99
2 Feb 1992
n=307
1 Jun 1992
n=220
4 Oct 1992
n=300
7 Feb 1993
n=251
1 Jun 1993
n=230
24 Nov 1991
n=199
1 Mar 1992
n=157
7 Jul 1992
n=250
1 Nov 1992
n=263
1 Mar 1993
n=146
4 Jul 1993
n=254
15 Dec 1991
n=65
1 Apr 1992
n=120
9 Aug 1992
n=191
1 Dec 1992
n=165
4 Apr 1993
n=238
1 Aug 1993
n=273

before any definite conclusions can be drawn. Unfortunately in April-May 1993 no specimens could be collected in Tapak, so that no data are available for April in the two consecutive years.

Seasonality within the Tapak population is somewhat different to that in Wedung. In Tapak more spawned and spent individuals were encountered throughout the year (compare Figs. 14A to 15A), and more females have higher percentages of small and large oocytes (5-20μm and 45-65μm) than females in Wedung (Table 7). It can also be seen that from the onset of the rainy season (October 1991) until just after the middle of the rainy season (January 1992) both sexes in Wedung were ripening. Yet in Tapak more than 50% of the specimens had already spawned (compare Figs.14A to 15A). This pattern in Tapak and Wedung however, was not consistent with that in the subsequent year. In Bandengan, that was the time when spawning and redeveloping proceeded simultaneously achieving peak redeveloping on May 1992 and 1993 (Fig. 16A). Apart from the period in August-November 1991, the population in Tapak mainly consisted of males (75.5%). During December 1991 until July 1992 the number of male individuals was in balance with the females, i.e. 51.06% to 52.22%, but after August 1992 this scale shifted in favour of females which on average comprised 61.36% to 68.93% of the population (Fig. 18).

In the histograms of size frequency distribution for monthly samples (Fig. 20) it can be seen that during the spawning and spent stages, individual *A. antiquata* cannot be sexed macroscopically, e.g. September-November 1991, January-February 1992, July-November 1992, January 1993 and July-August 1993. However, in the redeveloping stages they could easily be distinguished as male or female (March-May 1992, March-June 1993). For populations of *A. granosa* in Wedung the sex of all specimens was visually

unidentified in August-September 1991, 1992 and July-August 1993. Unfortunately no sections were made to confirm the gonad condition in August-September 1991. Regarding oocyte density, there was evidence of distinct spawning in those months in the following year, albeit neither as heavy as the one in October-December 1991 nor that in June/August 1993. Sections of subsamples taken from the recruits (14-18mm) in August-September 1992 confirmed that most of them were male specimens and that these were in the different stages of redeveloping and spawning.

3.3.4. Condition index and pea crab infestation on *A. antiquata*

Figure 20 summarises the monthly composition of male, female and undifferentiated specimens at the three study sites. Most members of the population from October 1991 to July 1992 and October 1992 to June 1993 in Wedung were confidently identified as male or female from the appearance of their reproductive tissue; this implies that they were either ripe or in the early stages of spawning (Fig. 17). During the mid dry season, i.e. August-September in both 1991 and 1992 as well as in July-August 1993, the occurrence of large undifferentiated individuals in Wedung coincided with juvenile recruitment. The recruits were mainly males, and were in the early stage of redeveloping or spent stages. Meanwhile, specimens collected from the population in Tapak were much larger than those in Wedung, and mostly were easily determined as male or female. Only a few remained unidentified (Fig. 20B).

Condition index changes with size (Fig. 21) but more specifically with respect to the time of collection as shown in Figures 23-24 for *A. granosa* from the populations in Wedung and Tapak, and *A. antiquata* in Bandengan.

Figure 20: The distribution of male, female and undifferentiated individuals summarised from Figures 17-19 to show the high percentages of undifferentiated individuals during the mid dry season (July-September) in Wedung which do not appear in the monthly samples in Tapak. In Bandengan, the occurrence of undifferentiated individuals is related to spawning activities in both the dry and rainy seasons.

A. *A. granosa*, Wedung,
B. *A. granosa*, Tapak,
C. *A. antiquata*, Bandengan.

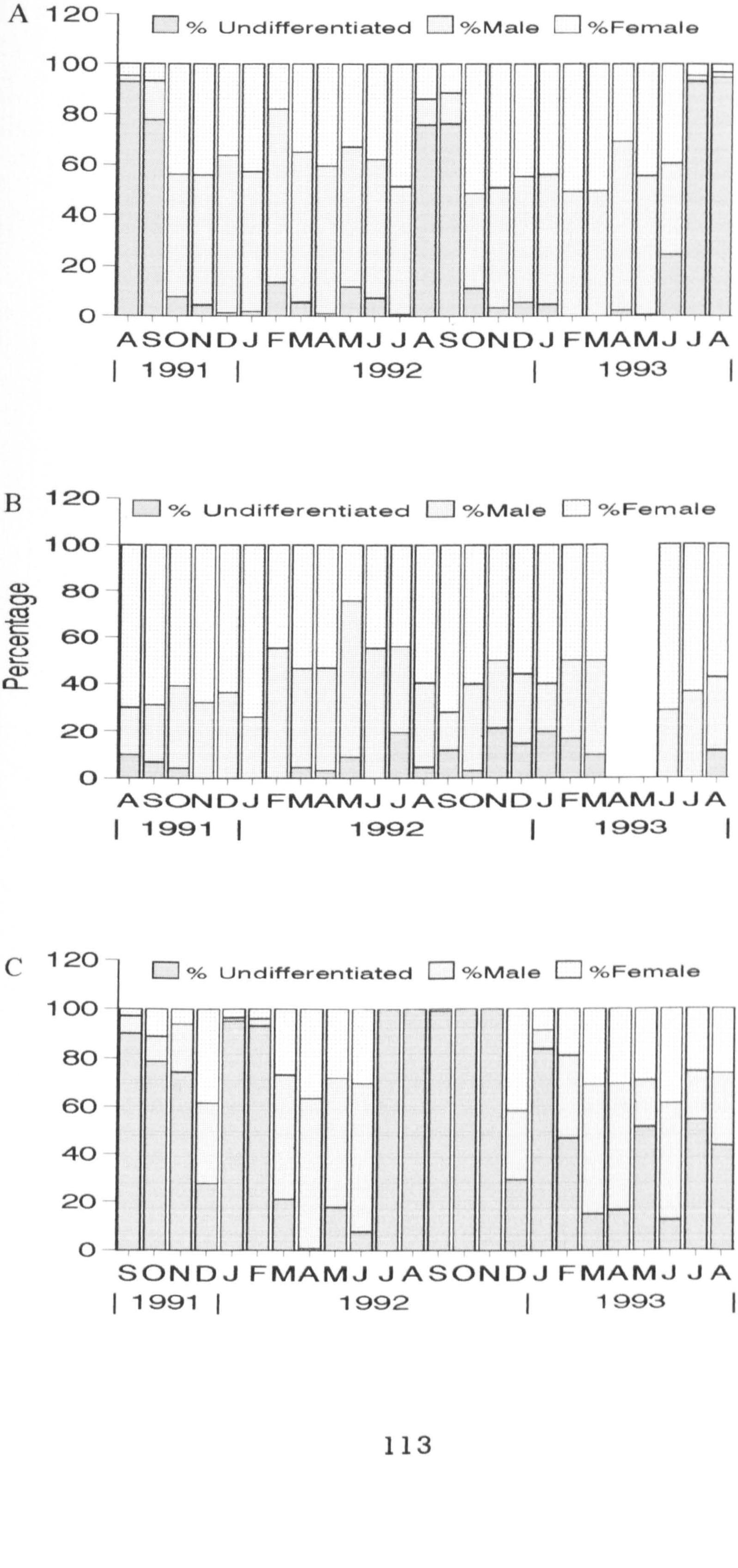

A
120
100
80
60
40
20
0
% Undifferentiated
%Male
%Female
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
Percentage
C

Figure 21: The overall relationship between Condition Index (CI) and Shell Length.

$$\mathbf{C.I} = \left[\frac{\text{Dry Tissue Weight}}{\text{Shell weight}}\right] \times 100$$

A. *A. granosa*, Wedung

B. *A. granosa*, Tapak

C. *A. antiquata*, Bandengan

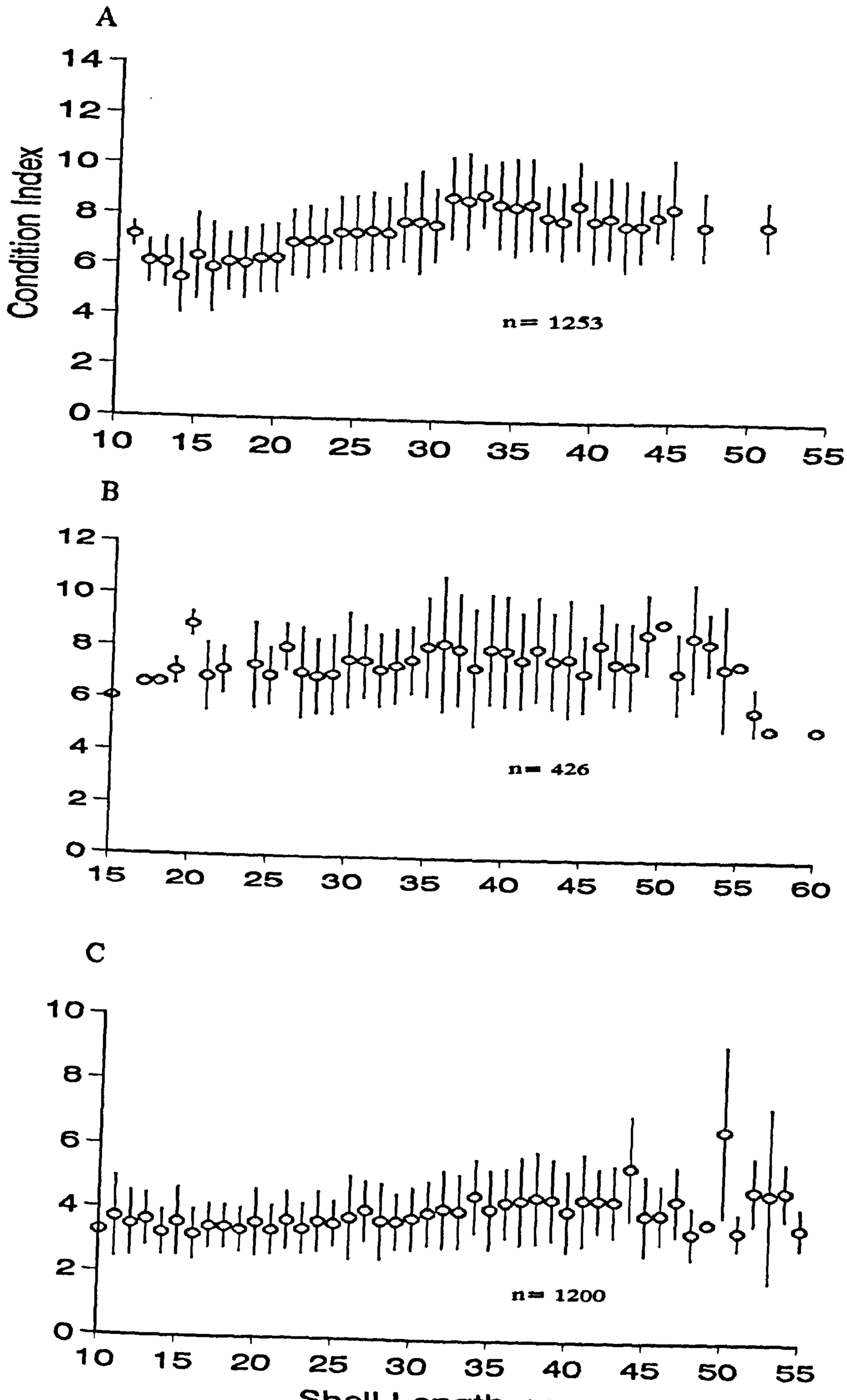

A
Condition Index
14
12
10
8
6
4
2
0
10 15 20 25 30 35 40 45 50 55
n= 1253
B
12
10
8
6
4
2
0
15 20 25 30 35 40 45 50 55 60
n= 426
C
10
8
6
4
2
0
10 15 20 25 30 35 40 45 50 55
n= 1200
Shell Length, mm

Figure 21 shows that up to a certain size (45mm onwards) condition indices in both *A. granosa* and *A. antiquata* were positively correlated to size. Thereafter, condition became less predictable but in general the condition index would then decline with further increase in size.

During the rainy season, most blood clams were easily determined as male or female as they were in the stages of developing gametes, whereas during the dry season their sex was macroscopically ill-defined as they were in the process of spawning. An analysis of covariance (Table 8D) using time of collection as a single covariate was applied to the condition indices of clams of comparable size in Wedung during the rainy season (February-April in both 1992 and 1993) and clams collected in certain months during the dry season (August-September 1992 and July-August 1993). The results thus reveal that although single regressions between the condition indices of clams in the rainy season (Table 8B) are higher than those for clams collected in the dry season (Table 8C, Fig. 22), the difference was not significant ($P>0.05$, Table 8D and Fig. 22).

The seasonal changes in the condition indices can only be constructed for *A. granosa* in Wedung and *A. antiquata* from Bandengan (Figures 23 and 24 respectively). This analysis was not possible for the Tapak population where too few specimens were available for collection. Subsequently, the regression constants as well as the correlation coefficients between a) condition indices and b) dry tissue weight to shell length for *A. granosa* and *A. antiquata* are presented in Tables 9 and 10 respectively.

The dry tissue weight has been correlated to shell length using the allometric equation (Chapter IV) based on logarithmic transformed data. Not surprisingly the calculation resulted in excellent r values compared to the r

Table 8A: The descriptive statistics of comparable size *A. granosa* collected in the rainy season (February-April) and the dry season (July-September) for used in analyses presented in Tables 8B-D

Month of collection (1991-1993)	Average size, mm	SD	Size range mm	n
February-April	27.54	8.87	15.2-56.3	127
July-September 1991-1993	27.56	9.17	15.3-56.3	127

Table 8B: Anova table for regression analysis between condition index and shell length of *A. granosa* collected in the rainy season (February-April 1991- 1993)

Source	DF	SS	MS	F	P	r
Regression	1	94.691	94.691	44.97*	0.001	0.515**
Error	125	263.209	2.106			
T o t a l	126	357.899				

Table 8C: Anova table for regression analysis between condition index and shell length of *A. granosa* collected in the dry season (July-September 1991- 1993)

Source	DF	SS	MS	F	P	r
Regression	1	32.156	32.156	7.76 *	0.006	0.241*
Error	125	518.085	4.145			
T o t a l	126	550.241				

Table 8D: Anova table for analysis of covariance using month as a single covariate between condition index of comparable size *A. granosa* (Wedung) collected at different season of the year.

Source	DF	SS	MS	F	P	
Size	1	119.608	119.608	38.27	0.001	
Month	1	0.179	0.179	0.06	0.811	
Size*Month	1	9.307	9.307	2.98	0.086	ns
Error	250	781.293	3.125			
T o t a l	253					

Degree of significance: see Table 6 in page 66

Figure 22: The relationship between condition index and shell length of *A. granosa* of comparable size from the Wedung population during the rainy (February-April, full line, ●) and dry season (July-September, broken line, □) within two consecutive years. The covariance analysis proved that the difference is not significant (P>0.05).

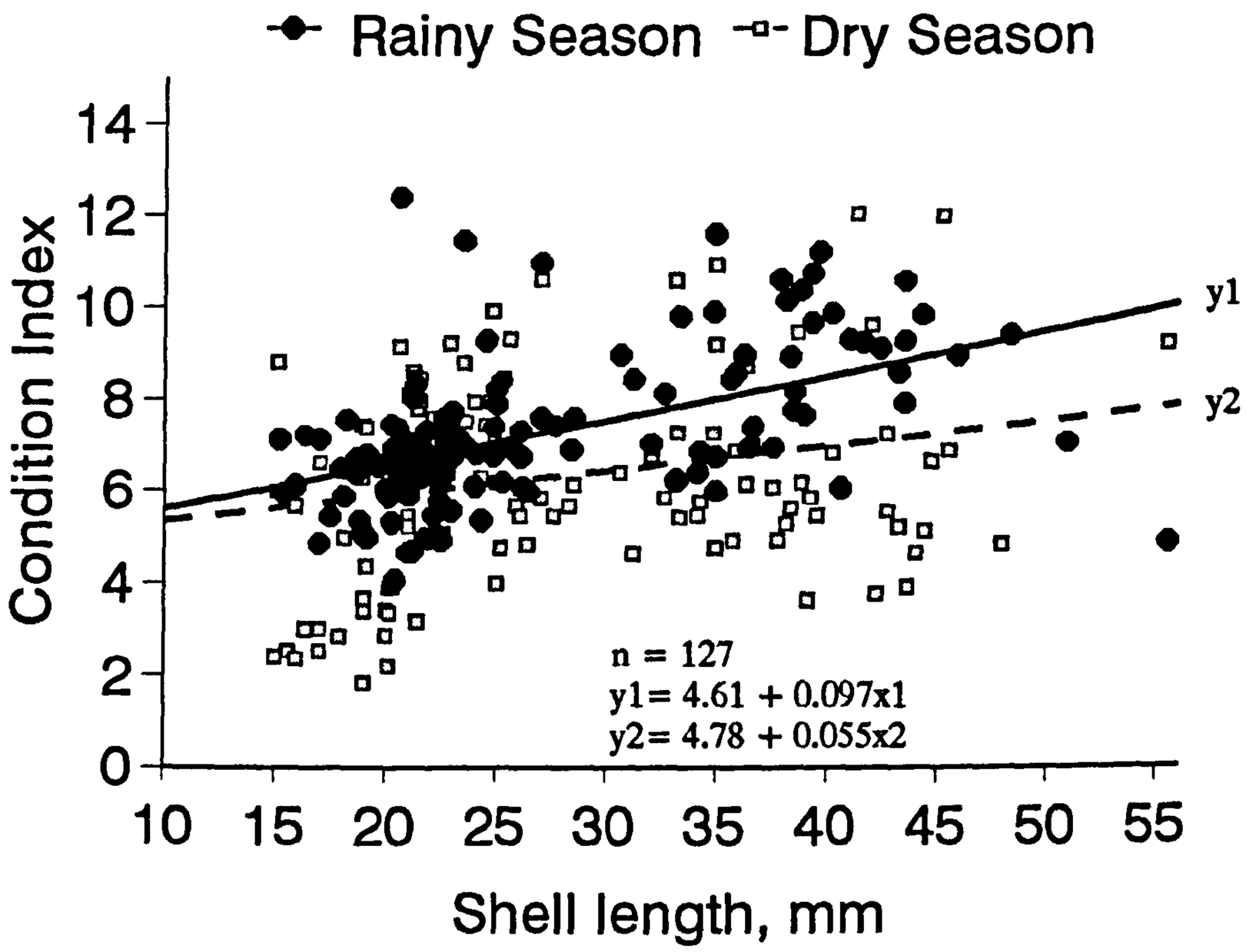

Rainy Season
Dry Season
Condition Index
14
12
10
8
6
4
2
0
y1
y2
n = 127
y1= 4.61 + 0.097x1
y2= 4.78 + 0.055x2
10
15
20
25
30
35
40
45
50
55
Shell length, mm

Table 9: Seasonal variability between condition index and shell length of monthly samples on *A. granosa* from Wedung, as well as for dry tissue weight-shell length (• denotes these regression constants in the equation $y= Ax^b$ where y is dry tissue weight as dependent variable and x is shell length as independent variable).

Month	a	b	r	a•	b•	r•
Aug 1991	-1.730	0.265	0.730***	$2.1E^{-07}$	4.28	0.933***
Sep 1991	-0.037	0.027	0.719***	$6.2E^{-06}$	3.35	0.962***
Oct 1991	10.700	0.122	-0.297*	$8.9E^{-05}$	2.51	0.897***
Nov 1991	4.830	0.145	0.311*	$2.3E^{-06}$	3.65	0.926***
Dec 1991	4.430	0.181	0.741***	$6.5E^{-06}$	3.34	0.994***
Jan 1992	0.530	0.338	0.787***	$6.0E^{-07}$	4.06	0.978***
Feb 1992	3.070	0.190	0.568***	$8.9E^{-06}$	3.22	0.961***
Mar 1992	2.290	0.169	0.677***	$9.3E^{06}$	3.15	0.982***
Apr 1992	2.080	0.220	0.528***	$2.5E^{-05}$	2.95	0.902***
May 1992	6.440	0.055	0.279 ns	$1.5E^{-05}$	3.06	0.974***
Jun 1992	3.950	0.132	0.542***	$8.5E^{-06}$	3.22	0.984***
Jul 1992	3.620	0.119	0.674***	$3.6E^{-05}$	2.80	0.972***
Aug 1992	4.090	0.144	0.791***	$1.0E^{-05}$	3.20	0.996***
Sep 1992	2.250	0.228	0.778***	$6.6E^{06}$	3.34	0.976***
Oct 1992	5.350	0.090	0.448***	$1.4E^{-05}$	3.07	0.980***
Nov 1992	4.870	0.139	0.566***	$1.2E^{-05}$	3.15	0.980***
Dec 1992	5.480	0.061	0.287 ns	$1.9E^{-05}$	2.97	0.958***
Jan 1993	5.580	0.035	0.302*	$2.6E^{-05}$	2.84	0.980***
Feb 1993	8.850	-0.052	-0.178 ns	$1.2E^{-04}$	2.42	0.915***
Mar 1993	8.730	0.002	0.009 ns	$1.4E^{-04}$	2.44	0.931***
Apr 1993	5.300	0.094	0.416**	$3.4E^{-05}$	2.83	0.964***
May 1993	3.040	0.102	0.490***	$1.3E^{-05}$	3.02	0.966***
Jun 1993	6.630	-0.040	-0.226 ns	$1.3E^{-04}$	2.31	0.941***
Jul 1993	6.690	-0.023	-0.285 ns	$9.1E^{-06}$	3.09	0.996***
Aug 1993	7.470	-0.044	-0.410**	$1.3E^{-05}$	2.96	0.994***

a and b are the intercept and the slope of the regression lines, and r is the coefficient of correlation (degree of significance: ns not significant, * $P<0.05$ ** $P<0.01$, *** $P<0.001$; df=48).

Table 10: Seasonal variability between condition index and shell length of monthly samples on *A. antiquata* from Bandengan, as well as for dry tissue weight-shell length (• denotes these regression constants in the equation $y= Ax^b$ where y is dry tissue weight as dependent variable and x is shell length as independent variable).

Month	a	b	r	a•	b•	r•
Sep 1991	1.940	0.046	0.552***	$3.3E^{-06}$	3.20	0.985***
Oct 1991	2.880	0.042	0.304*	$0.2E^{-05}$	2.77	0.927***
Nov 1991	-0.543	0.148	0.720***	$1.8E^{-07}$	4.10	0.957***
Dec 1991	1.660	0.096	0.555***	$1.9E^{-06}$	3.45	0.965***
Jan 1992	1.153	0.067	0.726***	$2.5E^{-06}$	3.28	0.985***
Feb 1992	2.340	0.041	0.426**	$2.9E^{-06}$	3.25	0.982***
Mar 1992	2.050	0.044	0.511***	$3.8E^{-06}$	3.16	0.986***
Apr 1992	4.762	-0.002	-0.013 ns	$9.5E^{-06}$	2.94	0.893***
May 1992	4.950	-0.025	-0.328*	$1.8E^{-05}$	2.76	0.984***
Jun 1992	6.560	-0.066	-0.583***	$2.5E^{-05}$	2.67	0.980***
Jul 1992	2.420	0.037	0.343*	$3.4E^{-06}$	3.20	0.978***
Aug 1992	3.130	0.026	0.241 ns	$4.4E^{-06}$	3.15	0.981***
Sep 1992	1.710	0.087	0.662**	$1.7E^{-06}$	3.45	0.986***
Oct 1992	4.758	-0.008	-0.061 ns	$5.8E^{-06}$	3.12	0.973***
Nov 1992	2.900	0.046	0.358*	$3.2E^{-06}$	3.27	0.976***
Dec 1992	13.400	-0.164	-0.645***	$1.2E^{-04}$	2.33	0.898***
Jan 1993	1.630	0.0719	0.605***	$2.0E^{-06}$	3.36	0.985***
Feb 1993	1.200	0.063	0.622***	$1.2E^{-06}$	3.50	0.980***
Mar 1993	0.190	0.128	0.566***	$1.6E^{-06}$	3.48	0.961***
Apr 1993	3.360	0.006	0.063 ns	$8.3E^{-06}$	2.97	0.964***
May 1993	3.000	0.006	0.095 ns	$1.3E^{-05}$	2.77	0.972***
Jun 1993	3.300	-0.007	-0.116 ns	$6.6E^{-06}$	2.96	0.982***
Jul 1993	2.180	0.026	0.322*	$4.0E^{-06}$	3.12	0.969***
Aug 1993	2.910	0.015	0.205 ns	$4.5E^{-06}$	3.13	0.983***

a and b are the intercept and the slope of the regression lines, and r is the coefficient of correlation (degree of significance: ns not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$; df=48).

values of the normal regression of condition index to shell length because the latter incorporated a second variable, i.e. shell weight. Furthermore, Figure 23 shows that the size range of *A. granosa* is not comparable from one month to another. Indeed some of the data are positively correlated e.g. August and September 1991, but some are negatively correlated albeit not significantly, e.g. June to August 1993, whilst others show almost no correlation such as in October 1991 and March 1993. This was similar for *A. antiquata* (Fig. 24). Therefore, in order to examine whether there were any seasonal variations in the relationship between condition index and size, only those samples in which there was a size range of clam between 17-34mm for *A. granosa* and 22-45mm for *A. antiquata* were included in the subsequent statistical analysis. This selection resulted in 12 and 20 monthly samples for each species respectively and these are denoted with an asterisk (*) in Figures 23-24.

Having analysed all the shell length data in the monthly samples, it is clear that the overall mean for the *A. granosa* population in Wedung is 26 mm, and 34 mm for *A. antiquata*. To allow a comparison of the condition index between the two species, then 30 mm as the mid-value was chosen as the standard sized animal.

Figures 26A and 27A however, relate the correlation coefficients calculated seasonally from all the 24 monthly samples for both species, where it is clear that dry tissue weight is always in positively and significant correlation to the condition index ($r = 0.932$ and $r = 0.725$ respectively, $P<0.002$, $df = 23$). In order to represent size ranges which may not be available in the monthly samples either due to the sampling technique or commercial harvest, the analysis for both species was carried out on the

Table 11: The Spearman rank order correlation between seasonal gonad index of the population, condition index and dry tissue weight of *A. granosa* and *A. antiquata* to the physical parameters monitored in Wedung, Tapak and Bandengan, October 1991-August 1993.

a. Population Gonad Index of monthly samples

	A. granosa	*A. antiquata*	
Parameters	Wedung	Tapak	Bandengan
Condition Index ♠	0.351 ns	n.a.	0.030 ns
Sediment Temperature	0.304 ns	-0.064 ns	0.015 ns
Chlorophyll-a	0.356 ns	-0.073 ns	-0.178 ns
Salinity	-0.291 ns	-0.246 ns	0.112 ns
Dissolved Oxygen	0.031 ns	0.186 ns	0.122 ns
Rainfall	0.110 ns	0.037 ns	0.046 ns

♠ Condition Index of 30mm standard size animal

b. Condition Index of 30 mm standard size animal

	A. granosa	*A. antiquata*
Parameters	Wedung	Bandengan
Dry Tissue Weight	0.932 ***	0.725 ***
Sediment Temperature	0.426 **	0.259 ns
Chlorophyll-a	0.511 ***	-0.055 ns
Salinity	-0.268 ns	0.060 ns
Dissolved Oxygen	0.231 ns	0.297 ns
Rainfall	0.381 ns	0.134 ns

c. Dry Tissue Weight of 30 mm standard size animal

	A. granosa	*A. antiquata*
Parameters	Wedung	Bandengan
Population Gonad Index	0.389 ns	0.153 ns
Sediment Temperature	0.309 ns	0.284 ns
Chlorophyll-a	0.462 **	0.095 ns
Salinity	-0.175 ns	-0.147 ns
Dissolved Oxygen	0.164 ns	0.256 ns
Rainfall	0.283 ns	0.029 ns

Degree of significance see Table 6 page 66, df= 23; n.a.= data not available

Figure 23: Regression lines showing the relationship of Condition Index and Shell Length in monthly samples of *A. granosa* from Wedung. The regression constants and degree of significance are presented in Table 9.

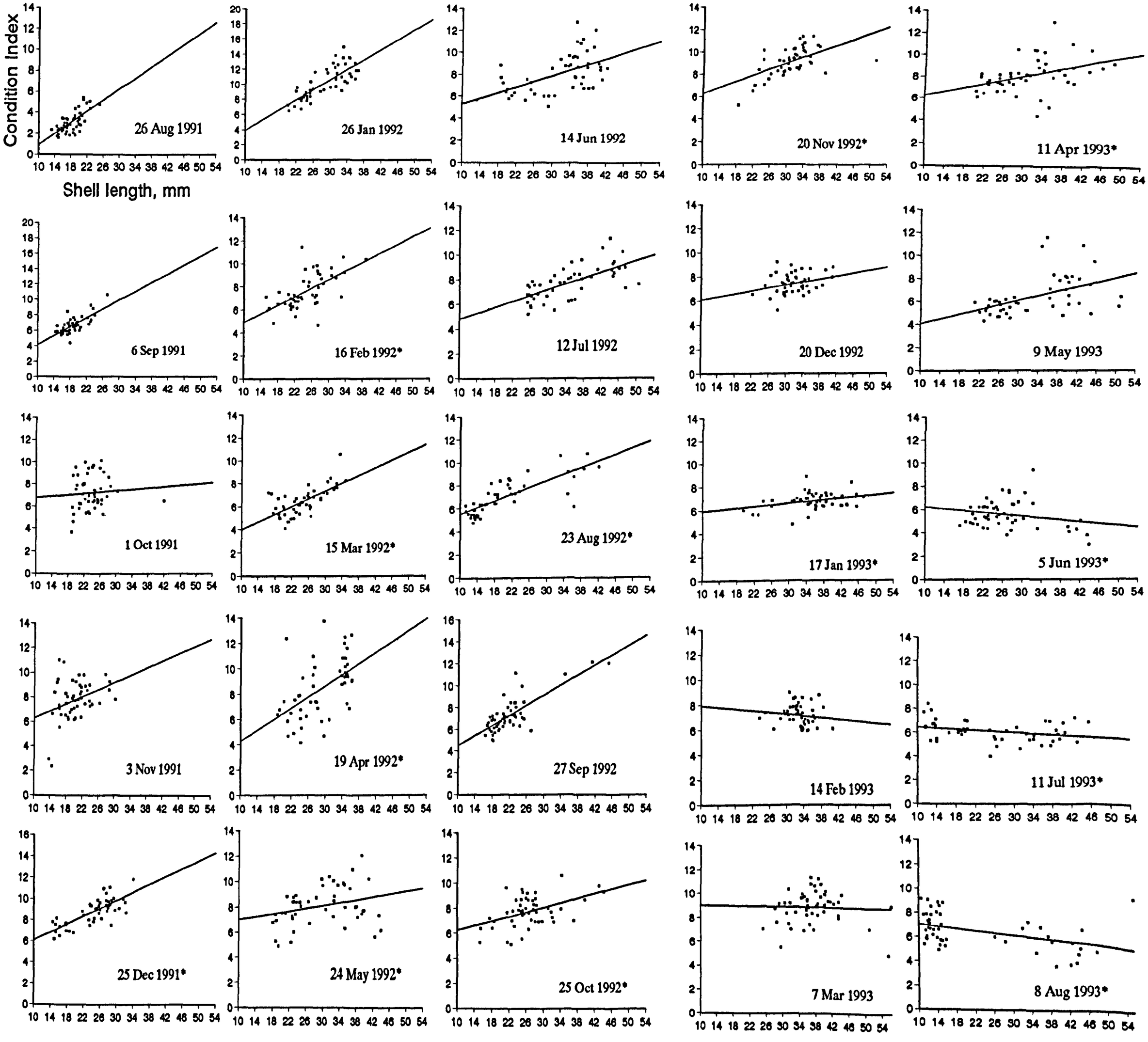
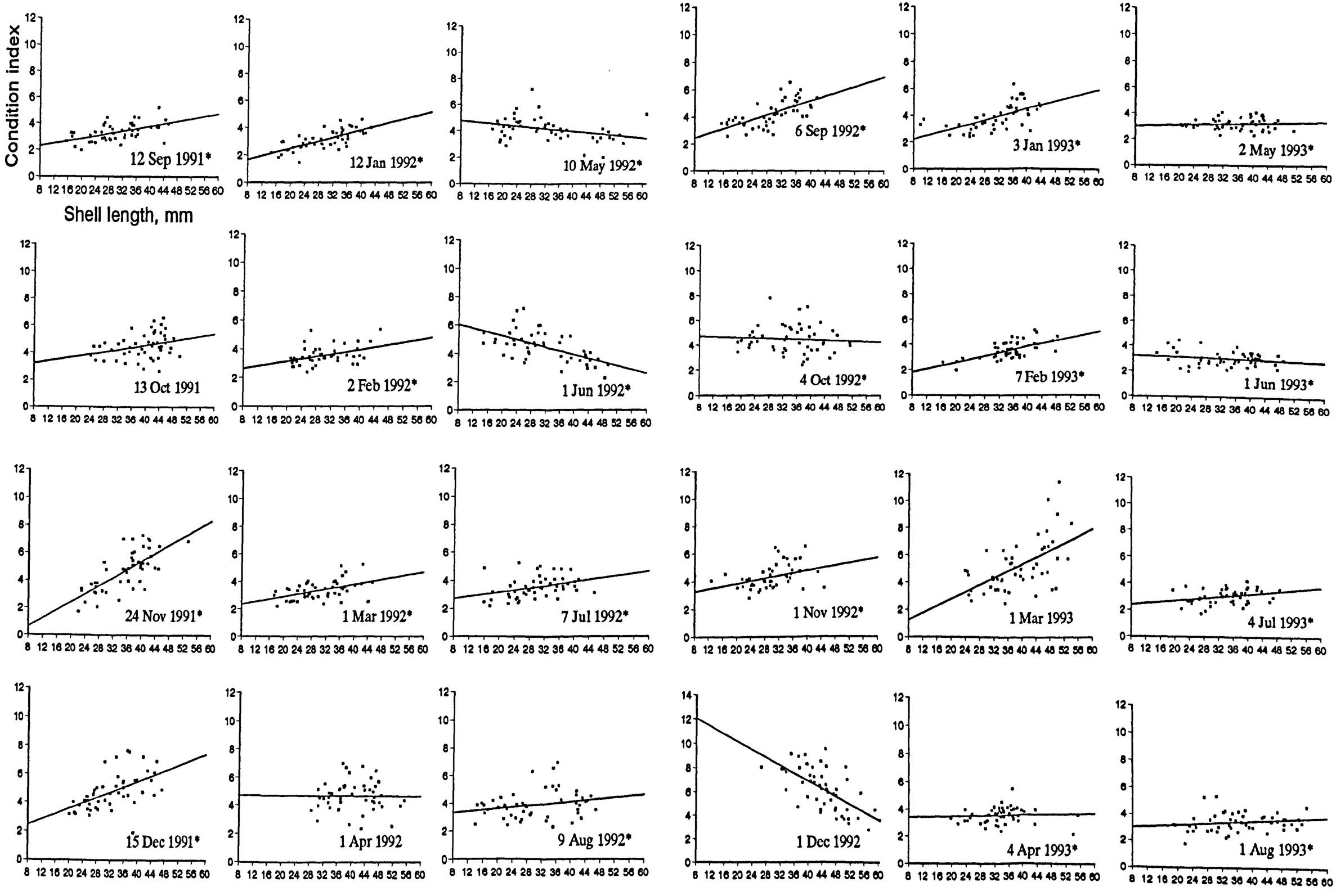

Condition Index
Shell length, mm
26 Aug 1991
26 Jan 1992
14 Jun 1992
20 Nov 1992*
11 Apr 1993*
6 Sep 1991
16 Feb 1992*
12 Jul 1992
20 Dec 1992
9 May 1993
1 Oct 1991
15 Mar 1992*
23 Aug 1992*
17 Jan 1993*
5 Jun 1993*
3 Nov 1991
19 Apr 1992*
27 Sep 1992
14 Feb 1993
11 Jul 1993*
25 Dec 1991*
24 May 1992*
25 Oct 1992*
7 Mar 1993
8 Aug 1993*

Figure 24: Regression lines showing the relationship of Condition Index and Shell Length in monthly samples of *A. antiquata* from Bandengan. The regression constants and degree of significance are presented in Table 10.


Condition index
Shell length, mm
12 Sep 1991*
12 Jan 1992*
10 May 1992*
6 Sep 1992*
3 Jan 1993*
2 May 1993*
13 Oct 1991
2 Feb 1992*
1 Jun 1992*
4 Oct 1992*
7 Feb 1993*
1 Jun 1993*
24 Nov 1991*
1 Mar 1992*
7 Jul 1992*
1 Nov 1992*
1 Mar 1993
4 Jul 1993*
15 Dec 1991*
1 Apr 1992
9 Aug 1992*
1 Dec 1992
4 Apr 1993*
1 Aug 1993*

30mm standard size animals for pairs of growth parameters (Table 11), except for the population gonad index which derived from monthly samples data. Accordingly, Figure 26 for *A. granosa* and Figure 27 for *A. antiquata* illustrate the seasonal variation between those growth as well as the physical parameters compared.

Table 11 indicates no significant agreement between the seasonal reproductive activities of *A. granosa* and certain environmental parameters, but some are significant in the case of physiological condition. The corresponding analysis cannot be made for the condition index in the Tapak population, again, due to the lack of available specimens. It is clear from Table 11 that some of the relationships appear to be significant only for the population of *A. granosa* in Wedung. In Tapak, none of those parameters compared indicate significant values (Table 11a). Apart from the dissolved oxygen concentration (r = 0.186) and monthly rainfall (r = 0.037), the rest were negatively related to the gonad index. This suggests that no linear relationships existed between the physiological/reproductive activities of the clams in Tapak and the surrounding physical environment.

Meanwhile in Bandengan, seasonal condition index and dry tissue weight of *A. antiquata* were both positively correlated to the oxygen availability in the water, but the seasonal gonad index for the population maintained a negative correlation with respect to the availability of food estimated as chlorophyll-a (r= -0.178). This indicates that seasonally, peaks of reproductive activities do not co-occur with the abundance of food, and population gonad index appeared to have no linear relation to either the condition index (r= 0.03), the dry tissue weight (r= 0.153) or the sediment temperature (r= 0.015). Yet when plotted together, the trend showed that 13

and 17 months out of 23 months for the condition index and the sediment temperature (Fig. 27C and D respectively) fluctuate simultaneously.

The population of *A. antiquata* in Bandengan however was, found to be infested by pinnotherid crabs. This pea crab was never found in *A. granosa* from Tapak and was observed only once in an isolated Wedung specimen. The sex ratio of the crabs is 1:6.5 in favour of females (Fig. 25C). The size range of the crab is 2-12mm, for which the average size of a male is 5mm and 7mm for the females.

During the two consecutive years of study, the level of occupancy by the crabs tends to increase starting from around February through to August and decreases from September to January (Fig. 25A), with the highest percentage (44.3%) of occupied clams occuring in May 1993.

Sex determination of the blood clams containing the crabs was carried out only by external examination of the reproductive tissue. This method is a rough guide to the sex and as such it was only possible to establish the gender of 24.90% of the infested clams. Nevertheless, the available data suggest the crabs do not exhibit any preference for male or female blood clams. The proportion of blood clams occupied was 1:1 (male 12.45% and female 12.45%) with the sex of 75.10% of the blood clams unidentified (Fig. 25D). The highest degree of occupancy was found in blood clams of 30-34 mm shell length although this only represented some 10% occupancy of the blood clams (Fig. 25B).

Figures 25E and F show the relationship between the size of male and female crabs respectively and the size of the blood clams occupied. The size of the infested females definitely increased with the size of the host clam ($r = 0.657$, $P<0.001$, $n= 209$) perhaps suggesting that occupancy of the blood clams started when both crabs and the blood clams were in their juvenile

Figure 25: The distribution of pea crab (pinnotherid) in *A. antiquata* from Bandengan.

A. The seasonal variation of pea crab occupancy.

B. The host size preferences for both sexes of pea crabs.

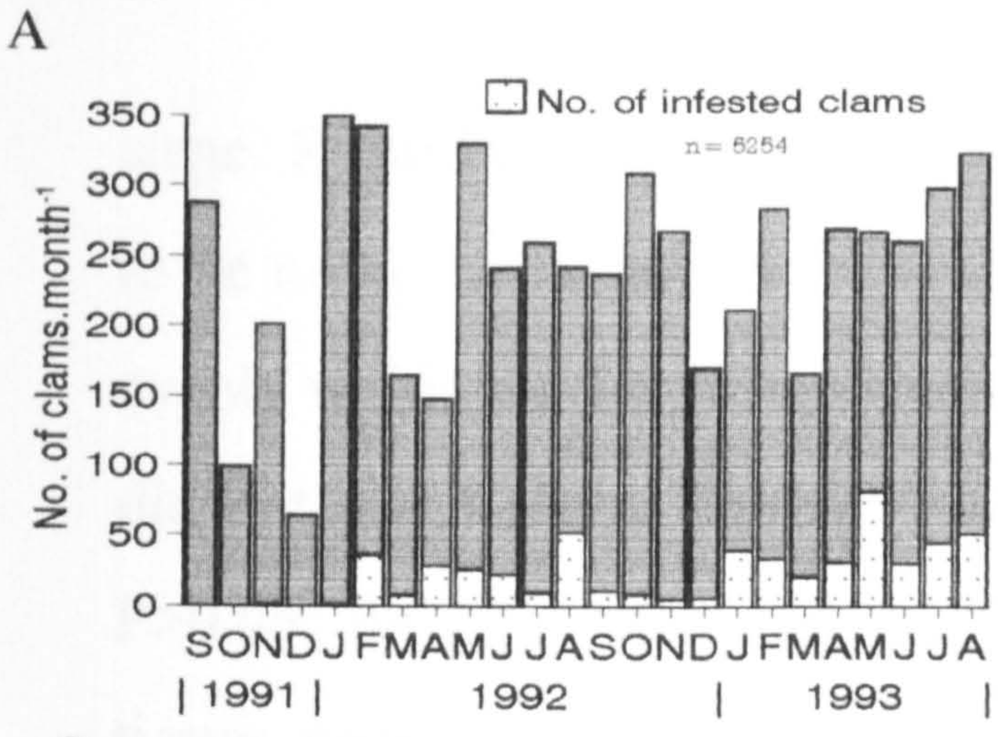
C. Distribution of male and female crabs occupying the size range of blood clams studied

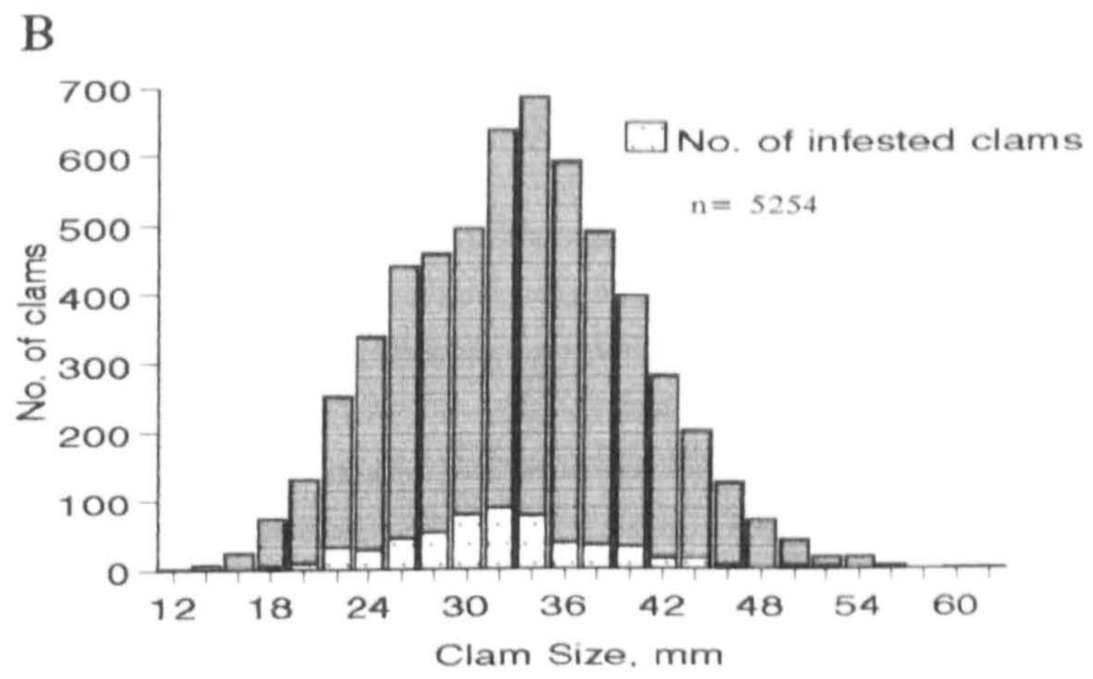
D. Size distribution of the blood clams, showing that 75.1% of the infested clams are unidentified.

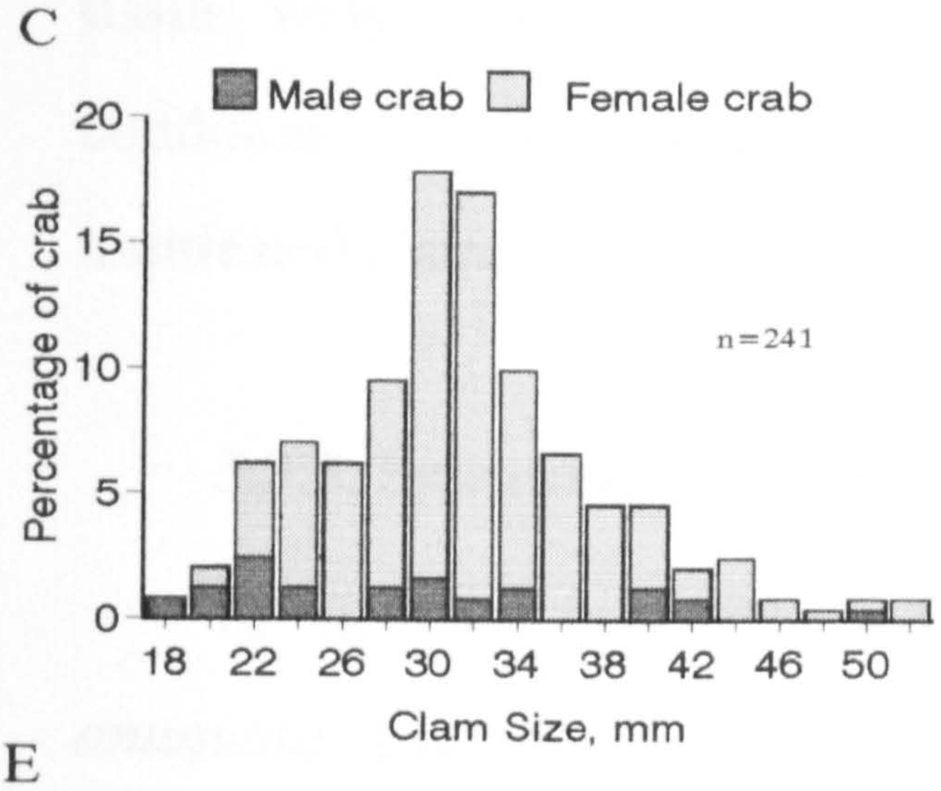
E. Correlation between size of male pea crabs and their host.

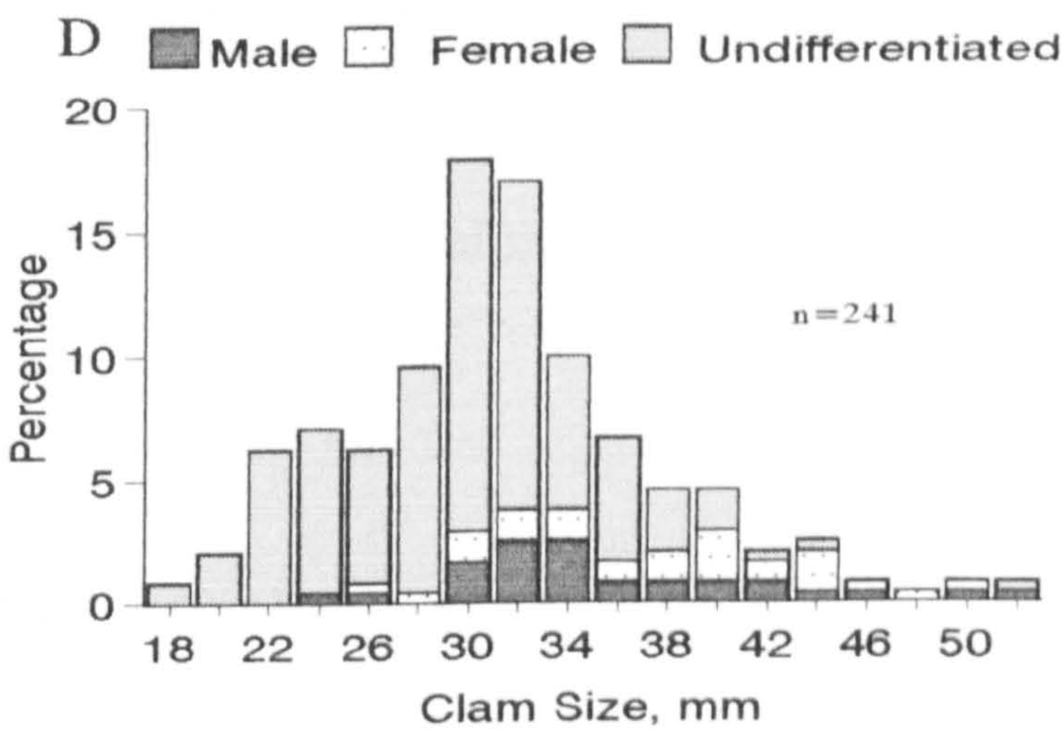
F. Correlation between size of female pea crabs (y) and their host (x). The regression line fitted the data in $y = 2.23 + 0.158x$ ($r=0.657$; $P<0.001$, $n= 209$).

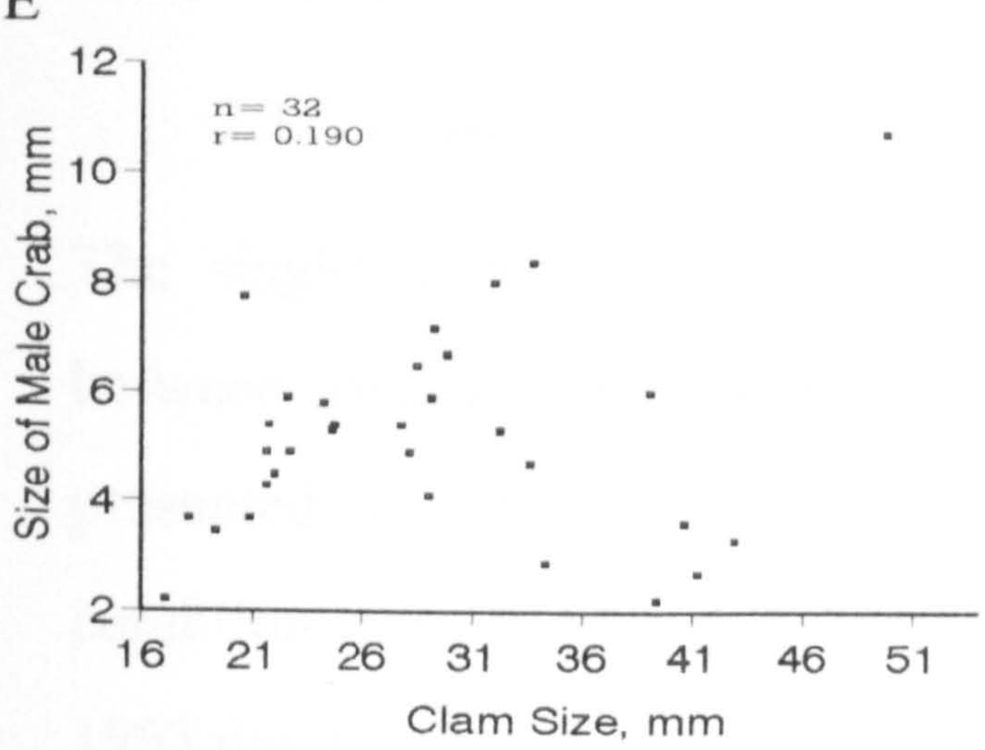
G. The regression lines of the dry tissue weight against shell length of infested clams $y = 7.2.10^{-6} + 2.93x$ (broken line, □) and non-infested clams $y = 4.2.10^{-6} + 3.16x$ (full line, O) of comparable size ranges. Further covariance analysis proved that their tissue weights differed significantly ($F= 6.37$, $0.012< P<0.02$, $n= 241$).

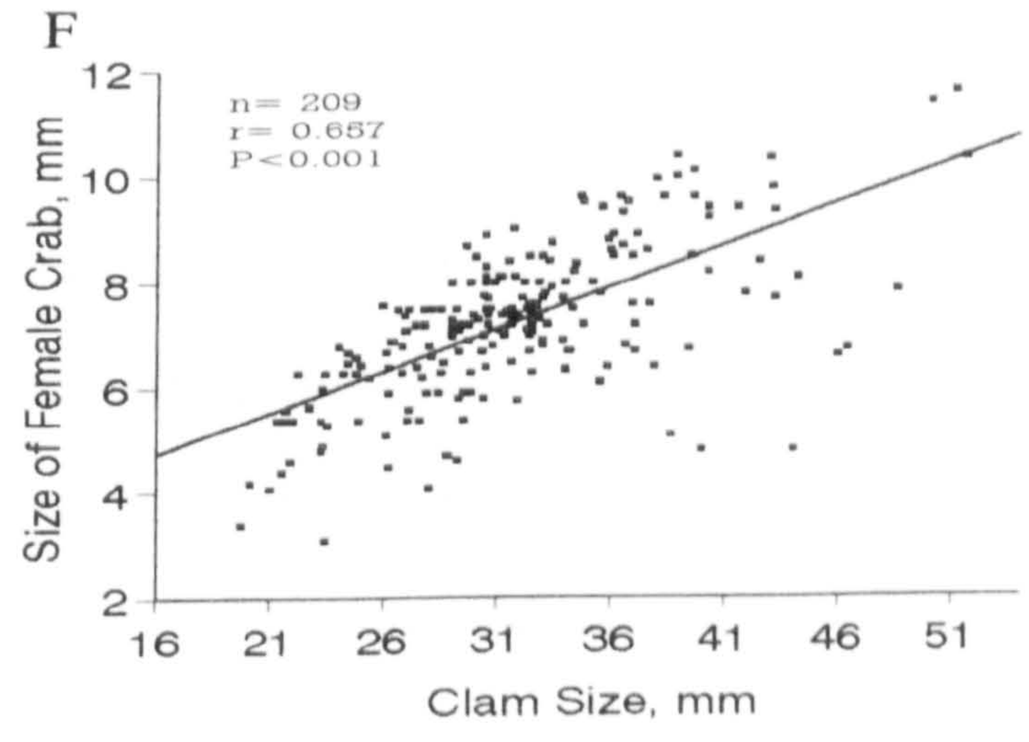

A
No. of infested clams
n= 5254
No. of clams.month-1
350
300
250
200
150
100
50
0
SONDJ FMAMJ JASONDJ FMAMJ JA
1991
1992
1993

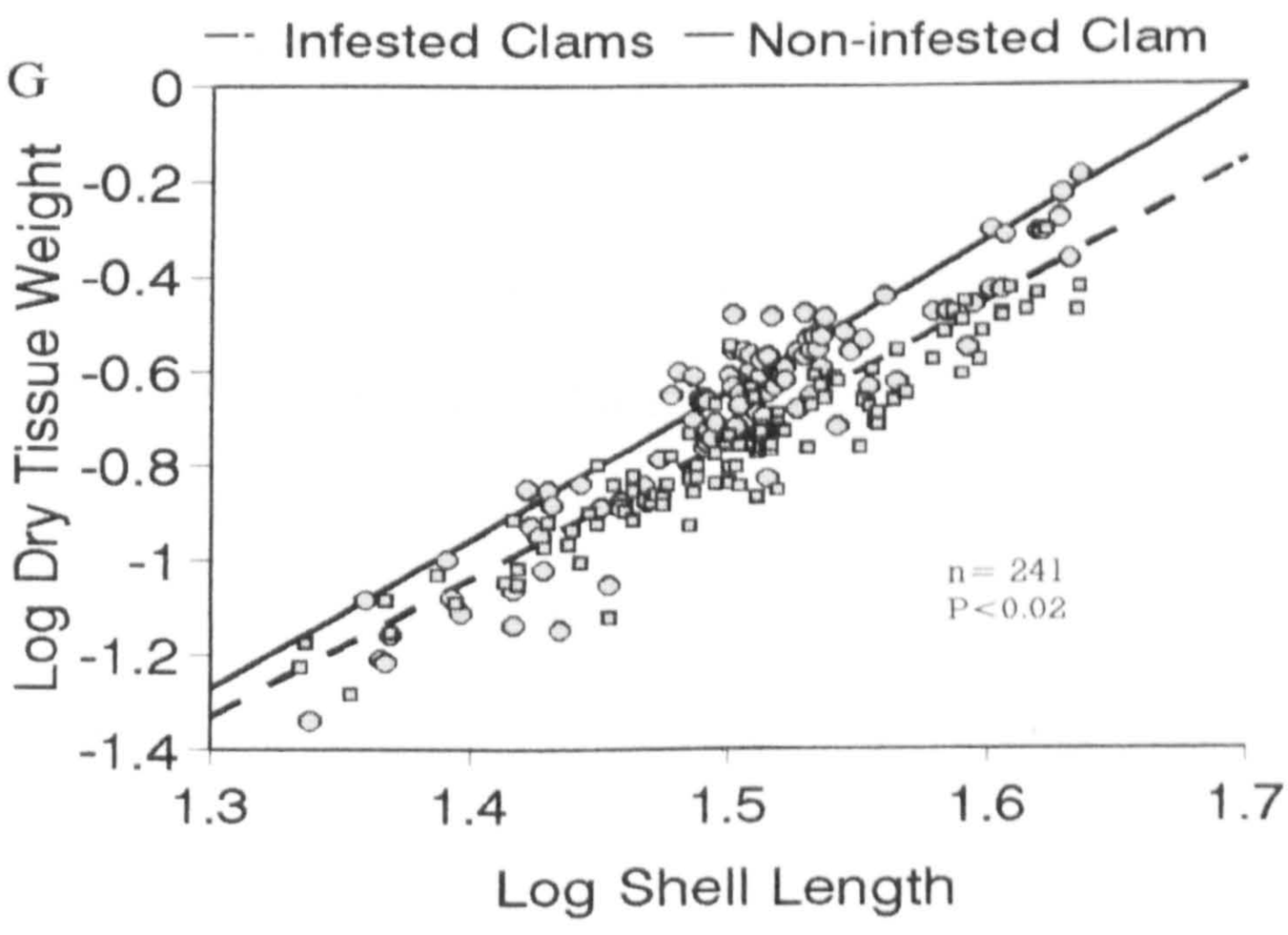

B
No. of infested clams
n= 5254
No. of clams
700
600
500
400
300
200
100
0
12 18 24 30 36 42 48 54 60
Clam Size, mm


C
Male crab
Female crab
Percentage of crab
20
15
10
5
0
n=241
18 22 26 30 34 38 42 46 50
Clam Size, mm


D
Male
Female
Undifferentiated
Percentage
20
15
10
5
0
n=241
18 22 26 30 34 38 42 46 50
Clam Size, mm


E
n= 32
r= 0.190
Size of Male Crab, mm
12
10
8
6
4
2
16 21 26 31 36 41 46 51
Clam Size, mm


F
n= 209
r= 0.657
P<0.001
Size of Female Crab, mm
12
10
8
6
4
2
16 21 26 31 36 41 46 51
Clam Size, mm


Infested Clams
Non-infested Clam
G
Log Dry Tissue Weight
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
n= 241
P<0.02
1.3 1.4 1.5 1.6 1.7
Log Shell Length

stage. Regardless of the small number of male crabs sampled, there appears to be no host size preference (Fig. 25E). The male crabs occurred relatively evenly within the 18-52 mm size range of clams and the size of the male crab did not significantly correlate with the size of the blood clams ($r= 0.190$, $P>0.05$; $n= 32$). Moreover, an analysis of covariance (GLM) comparing tissue weight between infested and uninfested clams revealed that the condition of crab-occupied clams was significantly poorer than those of the uninfested clams ($F= 6.37$; $n= 481$, $0.012>P>0.05$ for slope, Fig. 25G).

3.3.5. Spawning periodicity

In view of the differences that exist between *A. granosa* and *A. antiquata*, it is convenient to describe the spawning periodicity separately.

3.3.5.1. *Anadara granosa*

The single regression analyses carried out for assessing the relationship between condition index and size of the animal throughout the study period is presented in Table 9 for the Wedung population. The table outlines that condition index in May and December 1992, February, March, June and July 1993 did not change significantly according to the size of the animal ($P>0.05$, $df= 48$). Furthermore, the negative correlation showed in February, March and June 1993 was also not significant ($P>0.05$, $df= 48$).

Analysis of covariance using season as a single covariate applied to monthly samples of *A. granosa* which fall within the appropriate size range, showed there was a significant difference in the slope of the regression lines between condition index and shell length in 3 out of the 24 months ($F_{(11,454)}= 4.54$; $0.01>P>0.001$). The April 1992 and November 1992 samples, representing the beginning and the end of the rainy season in Wedung (Fig.7) had a significantly positive correlation compared with samples collected in

Table 12A: Anova table for analysis of covariance with a single covariate between condition index and size on the 12 selected monthly samples of *A. granosa*, Wedung.

Source	DF	SS	MS	F	P
Size	1	165.243	165.243	122.74	0.001
Season	11	47.924	4.357	3.24	0.001
Size*Season	11	67.286	6.117	4.54	0.001
Error	431	580.264	1.346		
T o t a l	454				

Table 12B: The departure of single regression slopes from the average slope as determined by analysis of covariance presented in Table 12a.

Term of regression	Coefficient	Std. Dev.	t- value	P
Constant	3.9501	0.3306	11.95	0.001
Size	0.1342	0.0121	11.08	0.001
Size*Season				
December 1991	0.04198	0.0435	0.97	0.335
February 1992	0.06698	0.0365	1.83	0.067
March 1992	0.05175	0.0348	1.49	0.137
April 1992	0.11709	0.0340	3.45	0.001
May 1992	0.04415	0.0408	1.08	0.280
August 1992	-0.04912	0.0291	-1.69	0.092
October 1992	-0.05160	0.0434	-1.19	0.235
November 1992	0.10539	0.0444	2.37	0.018
January 1993	-0.04921	0.0478	-1.03	0.304
April 1993	-0.04519	0.0405	-1.12	0.265
June 1993	-0.04406	0.0426	-1.03	0.302
July 1993	-0.18817	0.0410	-4.59	0.001

Figure 26: Seasonal pattern in the reproductive cycle of *A. granosa* from the Wedung population.

A. Correlation between condition index and size, calculated from the monthly samples.

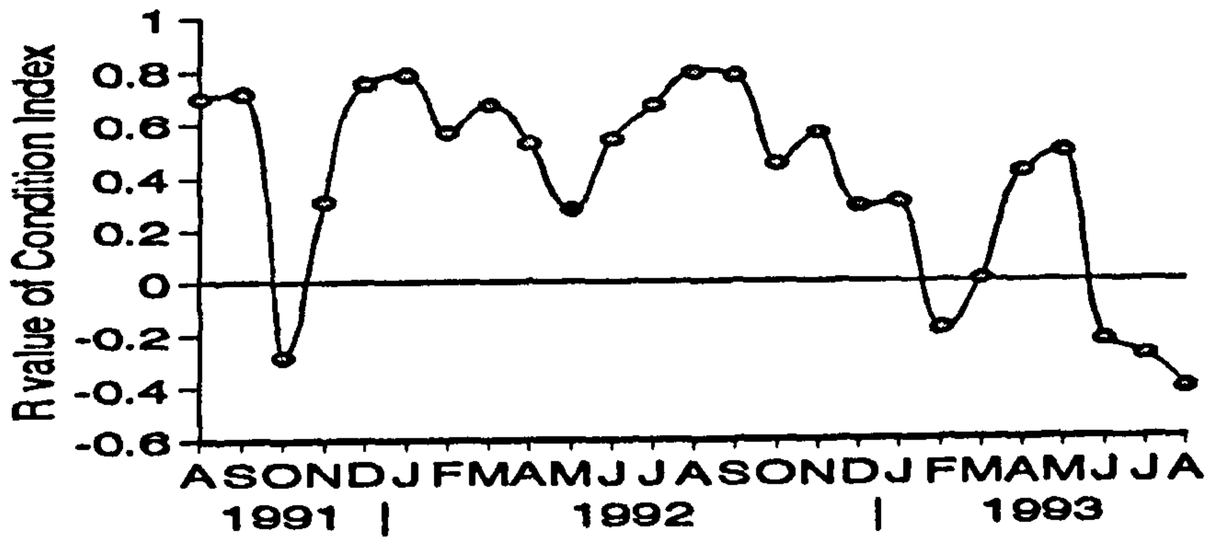
B. Seasonal variation between condition index and dry tissue weight of a 30 mm standard size *A. granosa.*

C. Seasonal variation between the condition index of a standard sized *A. granosa* of 30mm and gonad index derived from monthly samples. Changes in the mean gonad index during the study period calculated from the combination of both sexes

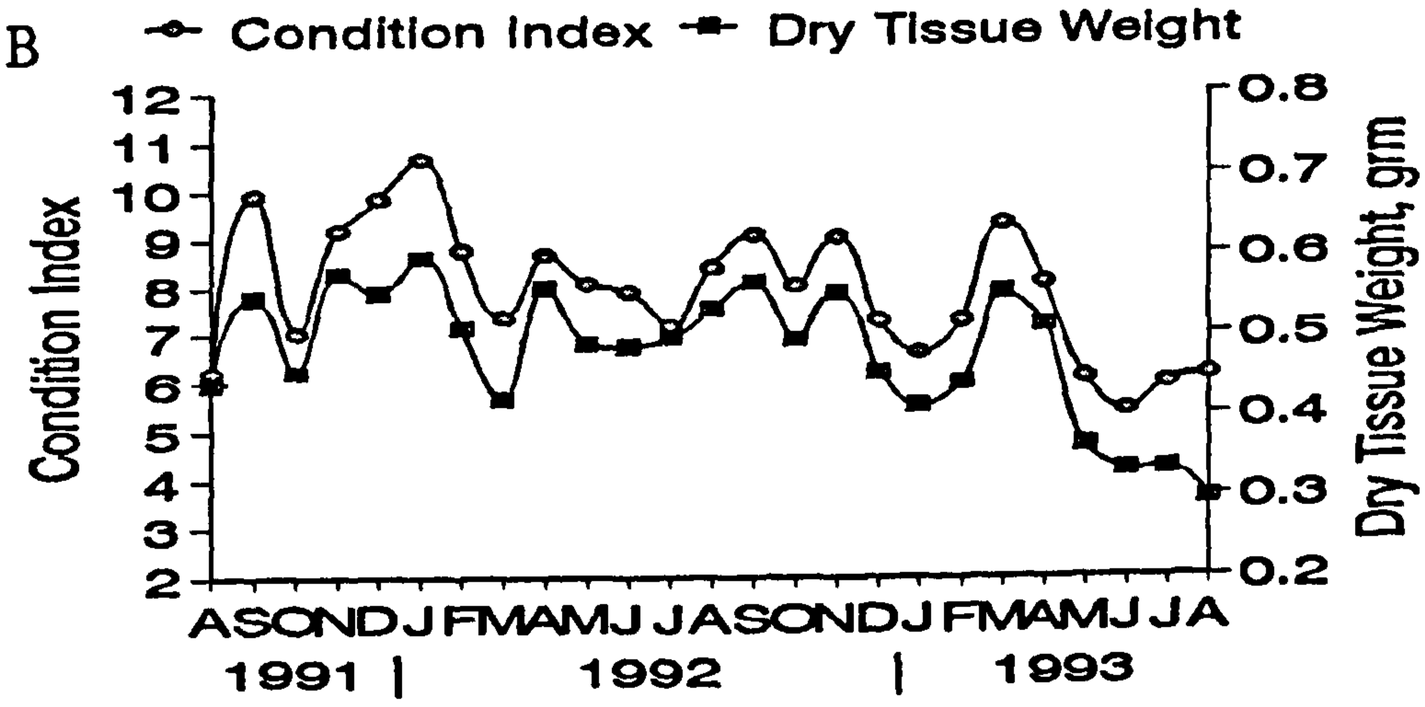
D. Seasonal variation for the relationship between the gonad index of individuals from the monthly samples and the sediment temperature.

E. Seasonal variation for the relationship between the condition index of standard sized *A. granosa* of 30mm and the sediment temperature.

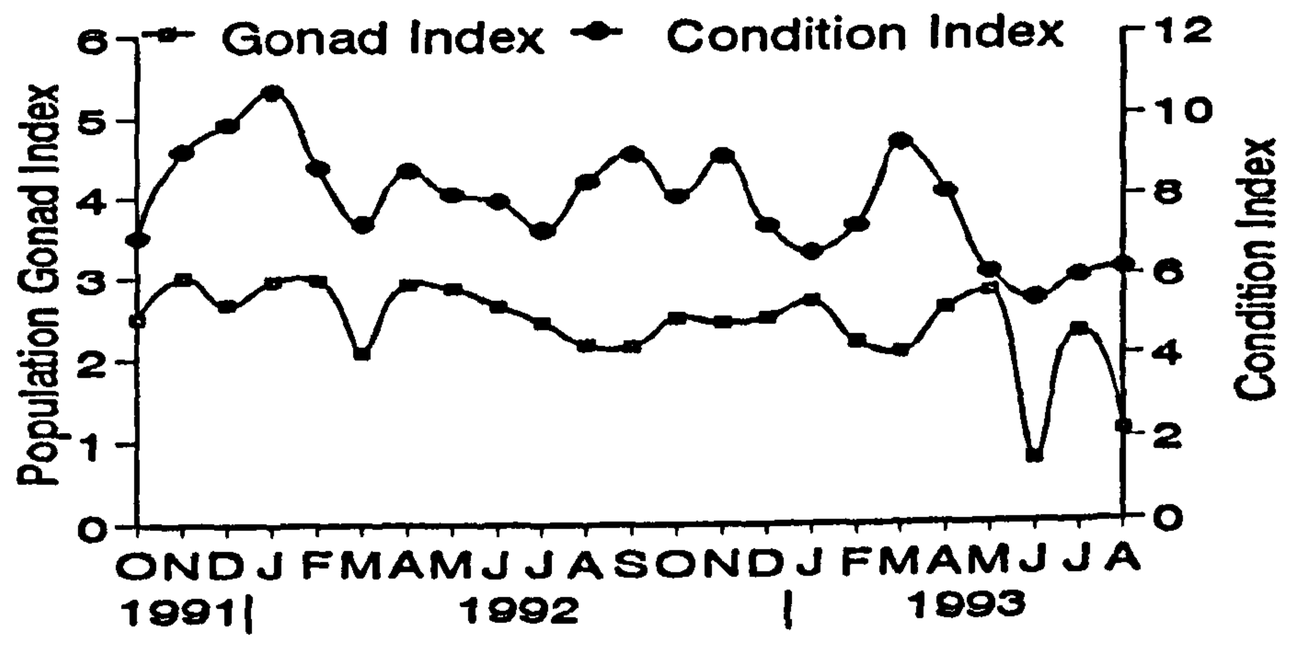
A


R value of Condition Index
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
ASONDJFMAMJJASONDJFMAMJJA
1991
1992
1993

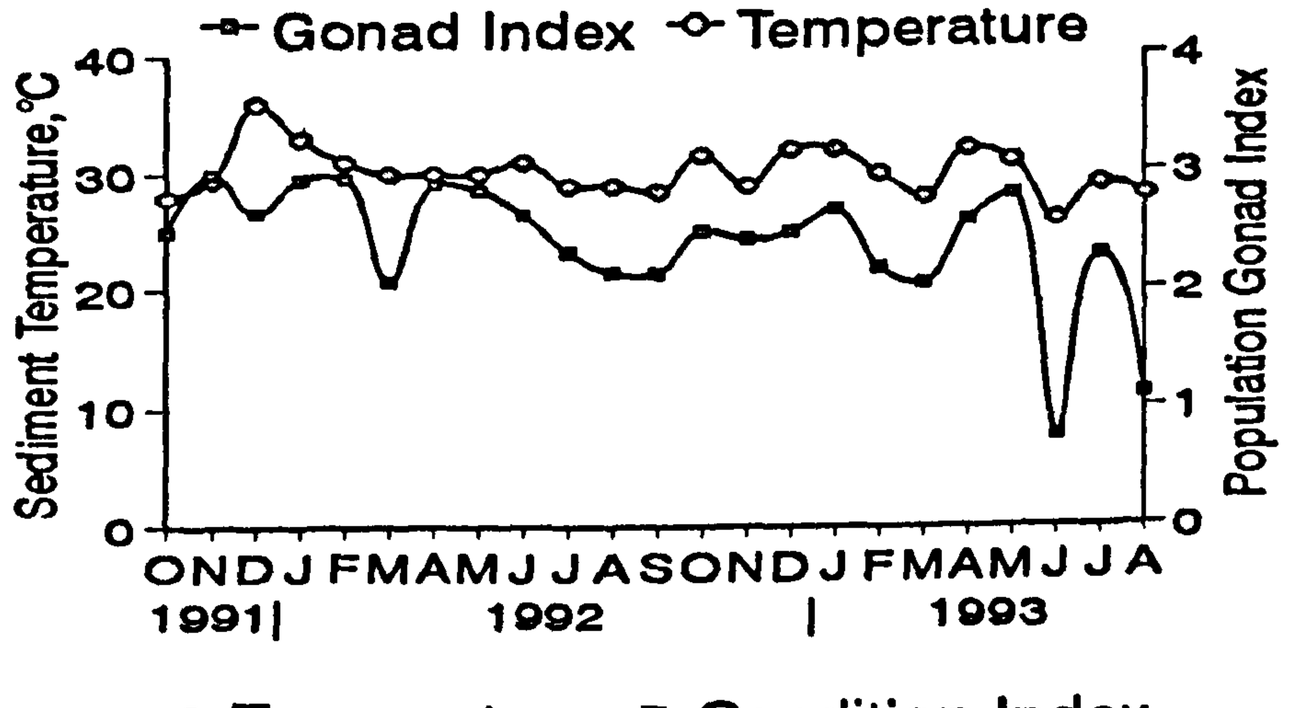
B


Condition Index
Dry Tissue Weight
Condition Index
Dry Tissue Weight, grm
12
11
10
9
8
7
6
5
4
3
2
0.8
0.7
0.6
0.5
0.4
0.3
0.2
ASONDJFMAMJJASONDJFMAMJJA
1991
1992
1993

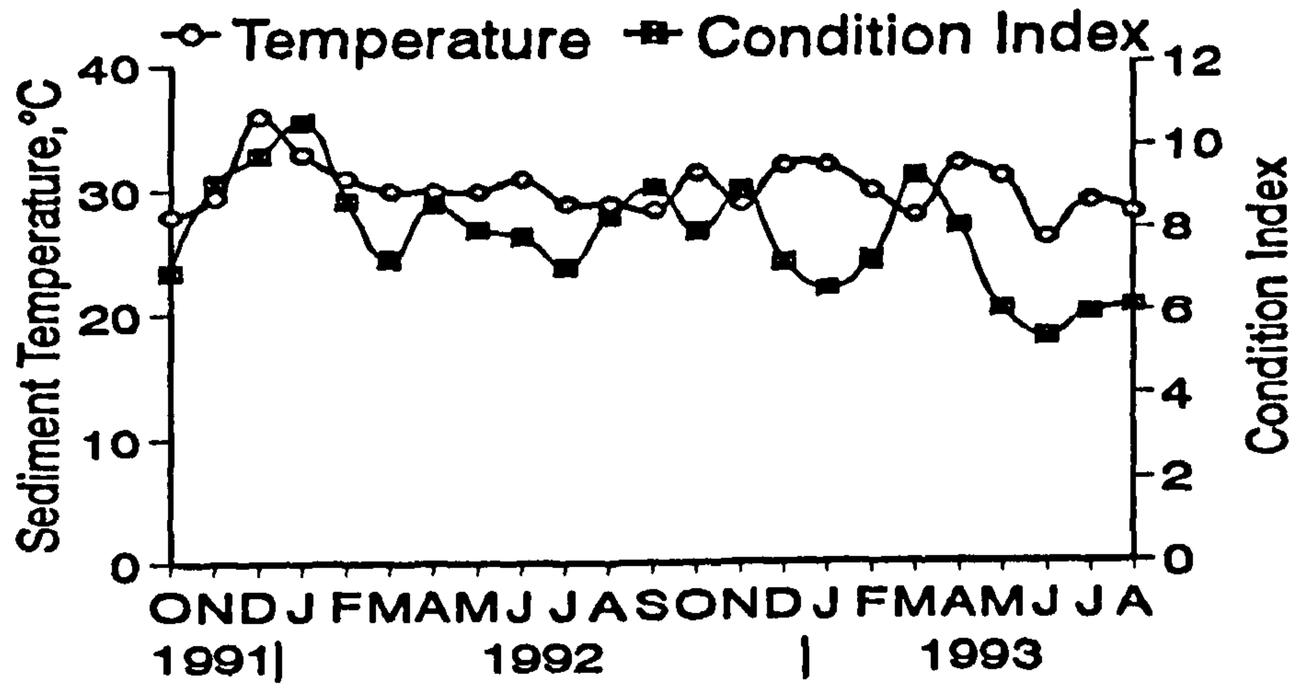
C


Gonad Index
Condition Index
Population Gonad Index
Condition Index
6
5
4
3
2
1
0
12
10
8
6
4
2
0
ONDJFMAMJJASONDJFMAMJJA
1991
1992
1993

D


Gonad Index
Temperature
Sediment Temperature,°C
Population Gonad Index
40
30
20
10
0
4
3
2
1
0
ONDJFMAMJJASONDJFMAMJJA
1991
1992
1993

E


Temperature
Condition Index
Sediment Temperature,°C
Condition Index
40
30
20
10
0
12
10
8
6
4
2
0
ONDJFMAMJJASONDJFMAMJJA
1991
1992
1993

July 1993 (dry season) which exhibit a negative correlation between the two parameters (Table 12, Figs. 23 and 26A).

In October 1991, the condition index and oocyte density dropped appreciably (Figs. 14C, 26A,B), but unfortunately this value cannot be compared to other months since the size range in this month did not fulfill the aforementioned size range criterion. This also occurred in January 1992 when condition index was at its highest. Nevertheless, a heavy spawning amongst female individuals in particular, has taken place sometime at the end of the dry season in Wedung and Tapak, i.e. October 1991, when the salinity was at its highest, temperature at its lowest, and whilst oxygen availability and chlorophyll-a concentration were relatively high.

In December 1991 the majority of the sections from the populations in Wedung and Tapak revealed that ripening was in progress. This occurred in the middle of the rainy season when the oxygen profile and chlorophyll-a concentrations were in steady state, salinity was markedly low (5‰ and 15‰) whilst the temperatures were quite high (36°C and 38°C in Wedung and Tapak respectively, Figs. 7-8 A & F). Subsequently at the beginning of the rainy season a further progress of ripening in both populations was followed by a minor spawning in Wedung which occurred late in January 1992 to mid February 1992. This was implied by a slight decrease in oocyte density combined with the lowest counts of small oocytes, whereas the medium to large size oocytes were maximal resulting in the highest gonad index. Similar values were obtained for male gonad indices (Fig. 14D). At this particular time of the year (i.e. rainy season), the condition index and the dry tissue weight of the monthly sample simulated by 30mm standard size animal were at their highest (Fig. 26B). This physiological feature coincides with certain environmental conditions when the oxygen saturation value was

higher compared to the preceeding and following months (Fig. 7D). Also the chlorophyll-a concentration was relatively high (Figs. 7-8E), the salinities in both sites were low (19‰ in Wedung and 27‰ in Tapak) and the temperature varied from second to the highest in Wedung (35°C, Figs. 7A,B) to normal (33°C, Figs. 8A,B) in Tapak.

In March 1992 there was an indication of redeveloping stages in the Wedung and Tapak populations. For the Wedung population, this was suggested following the remarkable decrease in physiological conditions such as the low values of the condition index, dry tissue weight (Fig. 26B) and shell growth increments (Fig. 14 and Fig. 36C in Chapter IV). In both populations this was also confirmed by the high density of small oocytes (Figs. 14-15A and C) which reflect the rather low values of female gonad index (Figs. 14-15D). At the same time, there was a slight increase in monthly rainfall in Wedung, whilst the percentage of oxygen saturation and the concentration of chlorophyll-a (Figs. 7-8 D & E) were at their highest during the year; salinity and temperature were 32‰ and 32°C for Wedung, and 30‰ and 40°C for Tapak respectively (Figs.7-8 A & B).

By April 1992, the slope of the regression line between the condition index and size in Wedung was positive and significant ($P \leq 0.001$, Table 12B) compared to other months. As in the preceeding month, the percentage of large eggs increased from 11.9 to 29.8%, resulting in the highest value for the female gonad index. In Tapak the proportion of oocyte distribution did not change from March the previous month (Fig. 15B), but the density indicated that some oocytes had been released (Fig. 15C). From the appearance of all males in the sections it was noted that some males were still ripening and this resulted in a rather low value for the male gonad index in both populations (Figs.14-15D). At this time, a further increase in the concentration of

chlorophyll-a during April 1992 (Figs. 7-8E), the decrease in salinity (17‰) and increase in temperature (35°C) resembled the conditions in January 1992 (Figs. 7A & B), when a minor spawning had taken place in Wedung. But these latter were not the case in Tapak where the salinity and temperature remained relatively normal compared to the previous month (30‰ and 35°C, Figs. 8A & B).

In Wedung, the female reproductive condition, in terms of oocyte density and distribution, was relatively constant until May 1992 indicating a period of redevelopment. In June 1992, the change in condition recorded at that time reflected a release and redevelopment of oocytes (Figs. 14 and 23). In Tapak however, a fully spent stage noted from only two female specimens collected in May 1992, suggests a major spawning followed by a rapid progress of redevelopment in June 1992 (Figs. 15A-C) whereas male specimens maintained a partially spawned condition as indicated by their lower gonad index (Fig. 15D).

In June 1992, male clams exhibited a higher gonad index than the females (Figs. 14-15D). Considering that the percentage of medium to large size eggs is among the highest but in a relatively lower density compared to June 1992, females in July must have been spawning (Figs. 14-15 A-C) and therefore physiological condition (Fig 26B), in terms of tissue weight was not as high as the previous and following months. There was also an indication of a distinct spawning sometime in this mid dry season between July-August 1992, because sections from mid-August indicated a progress of redeveloping oocytes.

The stage of female reproductive tissues remained relatively the same until September 1992, when the condition indices increased (Fig. 26B). In the same month the correlation coefficient between condition index and size

were among the highest (Fig. 26A). Environmental conditions in the driest month July 1992 in both Tapak and Wedung were about the same (Figs. 7-8 A & F). Indeed spawning was not as heavy as the one at the end of the dry season, October 1991, but it can be seen that shell growth increments at that particular time of the year (June-August 1992) decreased with respect to these reproductive activities (Fig. 14 and Fig. 36C in Chapter IV). Such a great variation in spawning could possibly explain the considerable year to year variability in the supply of spat available for on-growing cultivation.

Since a spawning had occurred in July-August 1992, larval settlement would probably take place over a month period (see Wong et al, 1985), i.e. sometime between September-October 1992 when food was abundant, as indicated by the highest concentration of chlorophyll-a (Figs. 7-8E). This may be interpreted as an evolved synchronisation of reproduction so that appropriate food is available for the larvae. On the other hand, the condition index of the spawners decreased (Fig. 26C) since more than 50% of the animals were in the redeveloping stage (Fig. 14A); so did the shell growth increments. However, in October and November 1992 the average increment of shell growth was higher than in September 1992 (Fig. 36C in Chapter IV) suggesting that the animals may channel some of their energy to somatic growth. In Tapak, soon after the spawners regain their reproductive tissue, i.e. when about 80% of them were in redeveloping stages in September 1992, they were again ready to spawn in October 1992 (Figs. 15A to D). This coincided with a minor increase in temperature and minor decrease in salinity (Figs. 8A & B).

From September 1992 onwards, reproductive stages in Wedung did not coincide with those in Tapak where they occurred two months earlier. So, when the spawners in Wedung ripened in November 1992 and were ready to

spawn by December 1992, in Tapak they were ripe in October 1992 and by December 1992 there were some spent individuals although the majority were in the redeveloping stages and these persisted until January 1993 (Figs. 14-15A and C).

Physiological condition again rose significantly in Wedung ($P<0.05$, Table 12B) in November 1992 when ripening follicles consisted mainly of medium to large oocytes. This again coincided with low salinity (17‰) and high sediment temperature (34°C). By the end of rainy season, April 1993, after a process of redevelopment in February-March 1993, spawning in female clams was more pronounced than males as indicated by the lower gonad index. Similar to the previous redevelopment stage in March 1992, in February 1993 the shell growth increments decreased following the progress of developing gametes (Fig. 14 and Fig. 36C in Chapter IV).

Having regained their reproductive material, followed by a further spawning and some redeveloping in May, there was a significant ($P<0.001$, Table 12B) spawning in July 1993. June to August 1993 can be considered to be the major spawning season for the *A. granosa* population in Wedung. At this mid dry season (June and August) there was an abrupt decline followed with a sudden increase in sediment temperature in July but compared to previous months during the study period, temperature during these particular periods were at their lowest.

The temperature variation during these months was paralleled by similar changes in the gonad index, condition index (Fig. 26, Table 11) and shell growth increments (Fig. 35 in Chapter IV), suggesting that temperature may be the main factor for triggering spawning. However, at this time the environmental conditions contrasted to those at the previous spawning time (November 1992) when high concentration of cholorophyll-a was combined

with low salinity and high temperature. Hence in June-August 1993 the chlorophyll-a concentration, temperature and the oxygen saturation values were at their lowest whilst salinities were amongst the highest (Fig. 7).

In July 1993, tissue weight as well as the condition index were relatively low, however, clams continued to redevelop their reproductive tissue for a subsequent major spawning in August 1993. Characteristically, sections of male individuals showed that none of them were completely spent, whilst the females varied from fully spent to fully ripe.

3.3.5.2. *Anadara antiquata*

In the two consecutive years, 1991-1993, female gonads of *A. antiquata* were active from November to July, with a ripe stage immediately emerging from a quiescent period each December (Fig. 16C). This latter seemed similar to that exhibited by *A. granosa* in December 1991 and December 1993 in Wedung and Tapak and July 1993 in Wedung (Fig. 14C). It occurred once in October 1992 when all the females were completely in the spent stage and males were in various stages of spawning (Fig. 16D). Visual examination of the external appearance of the reproductive tissue (Fig. 19) failed to determine the sex of the clams once they had started to spawn (Fig. 16), and therefore all of them were classified as undifferentiated. Only when their gonads were full or in developing/ ripening stages could clams be confidently sexed visually (e.g. March-July, Fig. 19). Only in 20 of the 24 monthly samples could *A. antiquata* of the size range 17-45mm may be found. Those samples which could not be included are October 1991, April 1992, December 1992 and March 1993. Analysis of covariance between condition index and size using season (month) as a single covariate revealed that as well as size ($F= 126.80$; $P<0.01$, $df_{(1, 943)}= 6.63$, Table 13A), season also has

Table 13A: Anova table for analysis of covariance running season as a single covariate between condition index and size on 20 selected monthly samples of *A. antiquata*, Bandengan.

Source	DF	SS	MS	F	P
Size	1	72.336	72.336	126.80	0.001
Season	19	133.833	7.044	12.35	0.001
Size*Season	19	130.958	6.893	12.08	0.001
Error	904	515.723	0.570		
T o t a l	943				

Table 13B: The departure of single regression slopes from the average slope as determined by analysis of covariance presented in Table 13a.

Term of regression	Coefficient	Std. Dev.	t- value	P
Constant	2.4682	0.1208	20.43	0.001
Size	0.04086	0.0036	11.26	0.001
*Size*Season*				
September 1991	0.0148	0.0159	0.94	0.350
November 1991	0.1118	0.0164	6.82	0.001
December 1991	0.0551	0.0144	3.82	0.001
January 1992	0.0302	0.0169	1.79	0.074
February 1992	-0.00006	0.0163	-0.00	0.997
March 1992	0.0073	0.0158	0.46	0.645
May 1992	-0.0799	0.0126	-6.34	0.001
June 1992	-0.1114	0.0123	-9.08	0.001
July 1992	-0.0029	0.0176	-0.17	0.868
August 1992	-0.0161	0.0135	-1.19	0.235

Table 13B: The departure of single regression slopes from the average slope as determined by analysis of covariance presented in Table 13a, (continued).

Term of regression	Coefficient	Std. Dev.	t- value	P
Constant	2.4682	0.1208	20.43	0.001
Size	0.04086	0.0036	11.26	0.001
*Size*Season*				
September 1992	0.0587	0.0184	3.20	0.001
October 1992	-0.0464	0.0136	-3.40	0.001
November 1992	0.0189	0.0181	1.04	0.297
January 1993	0.0579	0.0163	3.54	0.001
February 1993	0.0247	0.0178	1.39	0.165
April 1993	-0.0094	0.0203	-0.46	0.643
May 1993	-0.0313	0.0151	-2.07	0.038
June 1993	-0.0419	0.0138	-3.02	0.003
July 1993	-0.0153	0.0152	-1.01	0.315
August 1993	-0.0247	0.0132	-1.88	0.061

Degree of significance see Table 6 in page 66

Figure 27: Seasonal pattern in the reproductive cycle of *A. antiquata* from the Bandengan population.

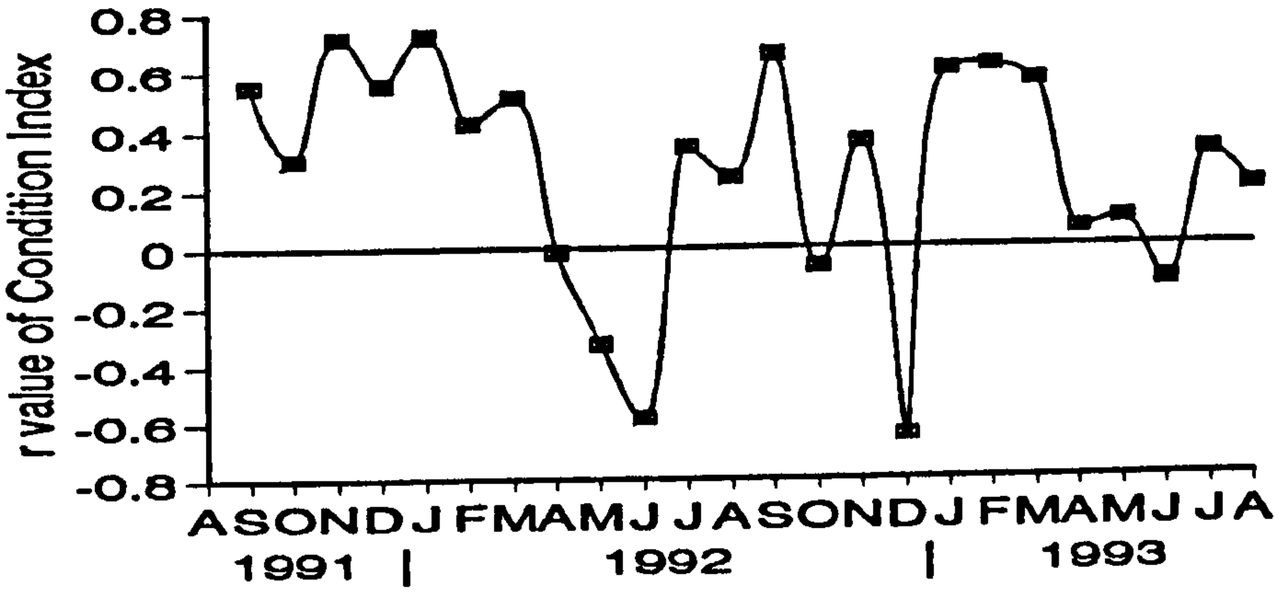
A. Correlation between condition index and size, calculated from the monthly samples.

B. Seasonal variation between the condition index and dry tissue weight of a 30 mm standard size *A. antiquata.*

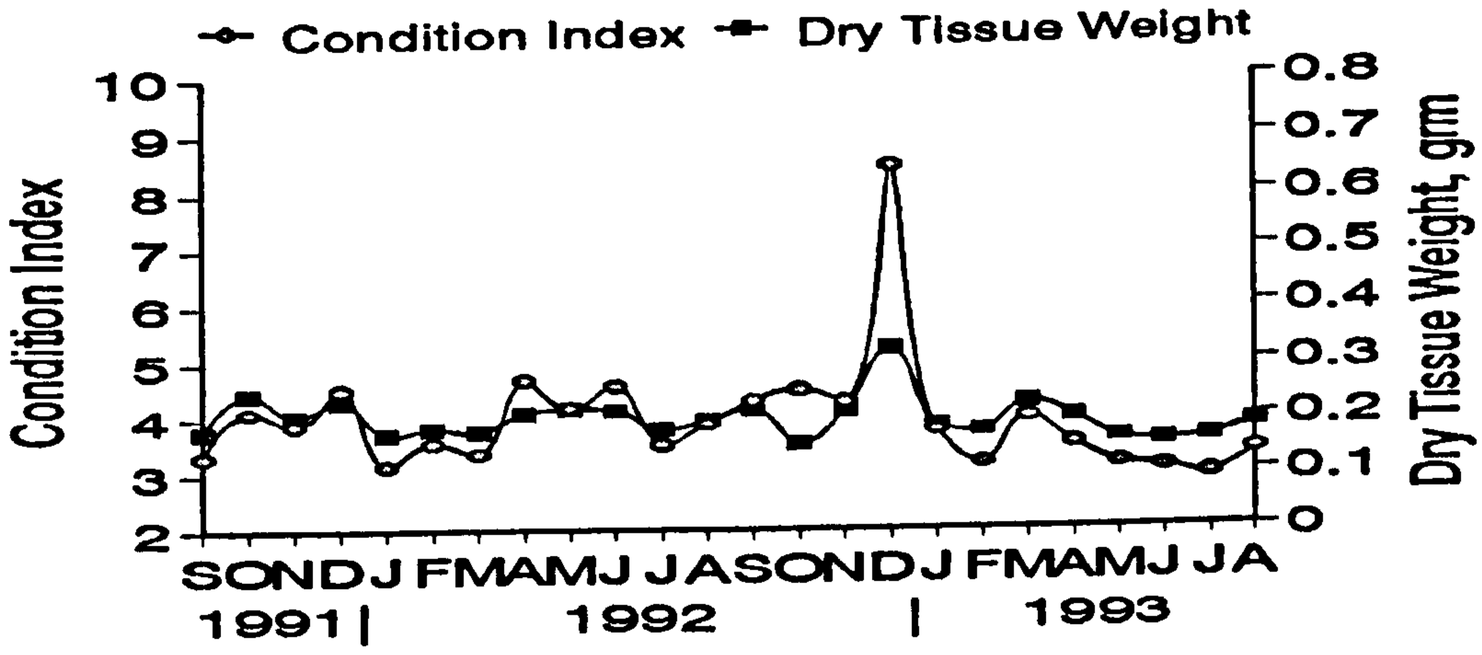
C. Seasonal variation between the condition index of a standard sized *A. antiquata* of 30mm and gonad index derived from monthly samples. Changes in the mean gonad index during the study period calculated from the combination of both sexes

D. Seasonal variation for the relations between the condition index of standard size *A. antiquata* of 30mm and the sediment temperature.

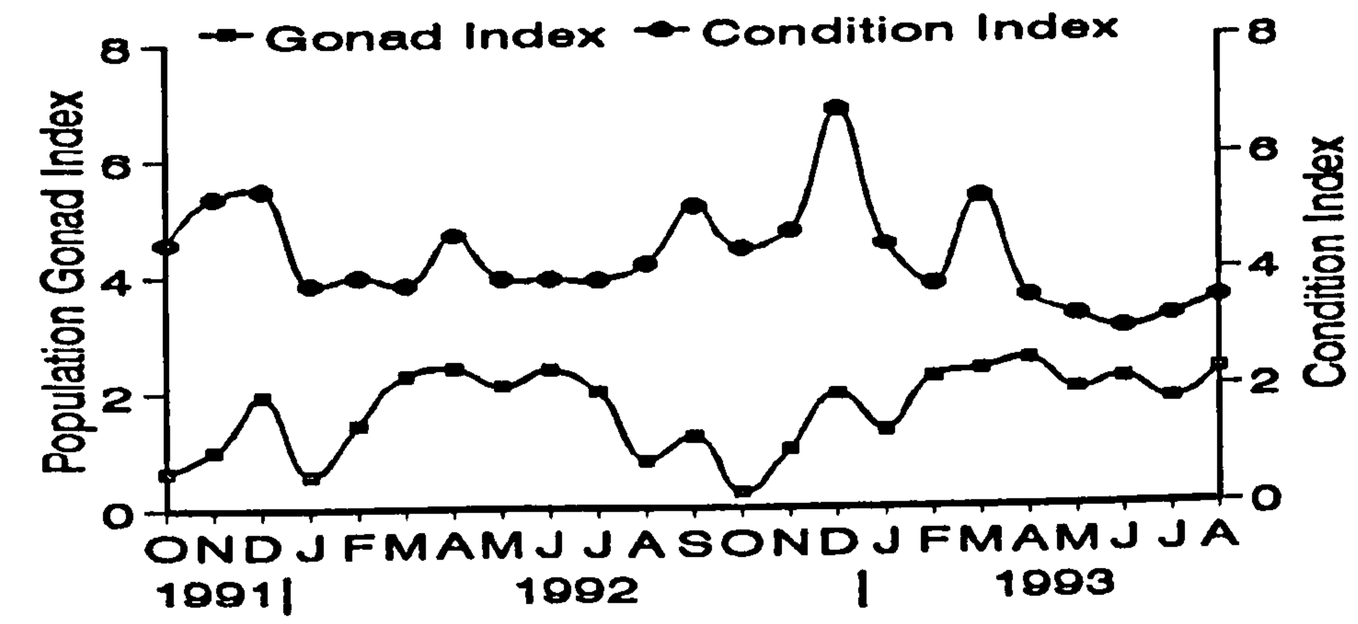
A


r value of Condition Index
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

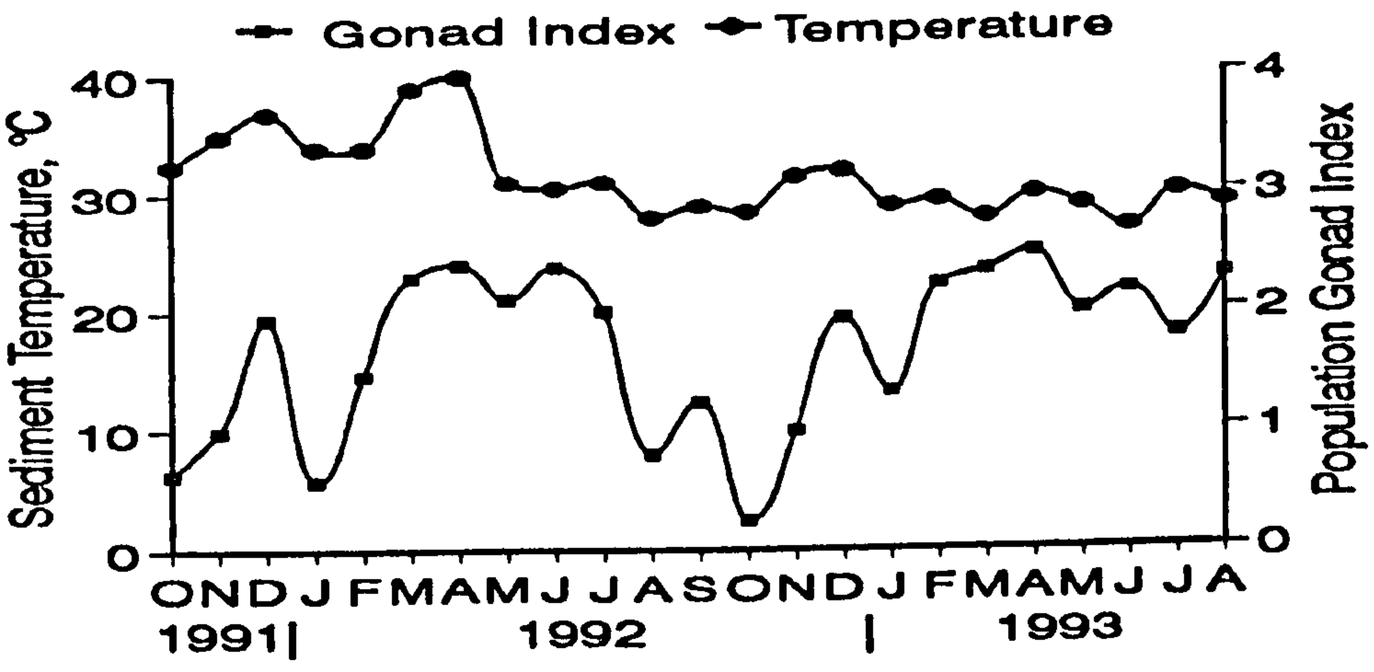
B


Condition Index
Dry Tissue Weight
Condition Index
Dry Tissue Weight, grm
10
9
8
7
6
5
4
3
2
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

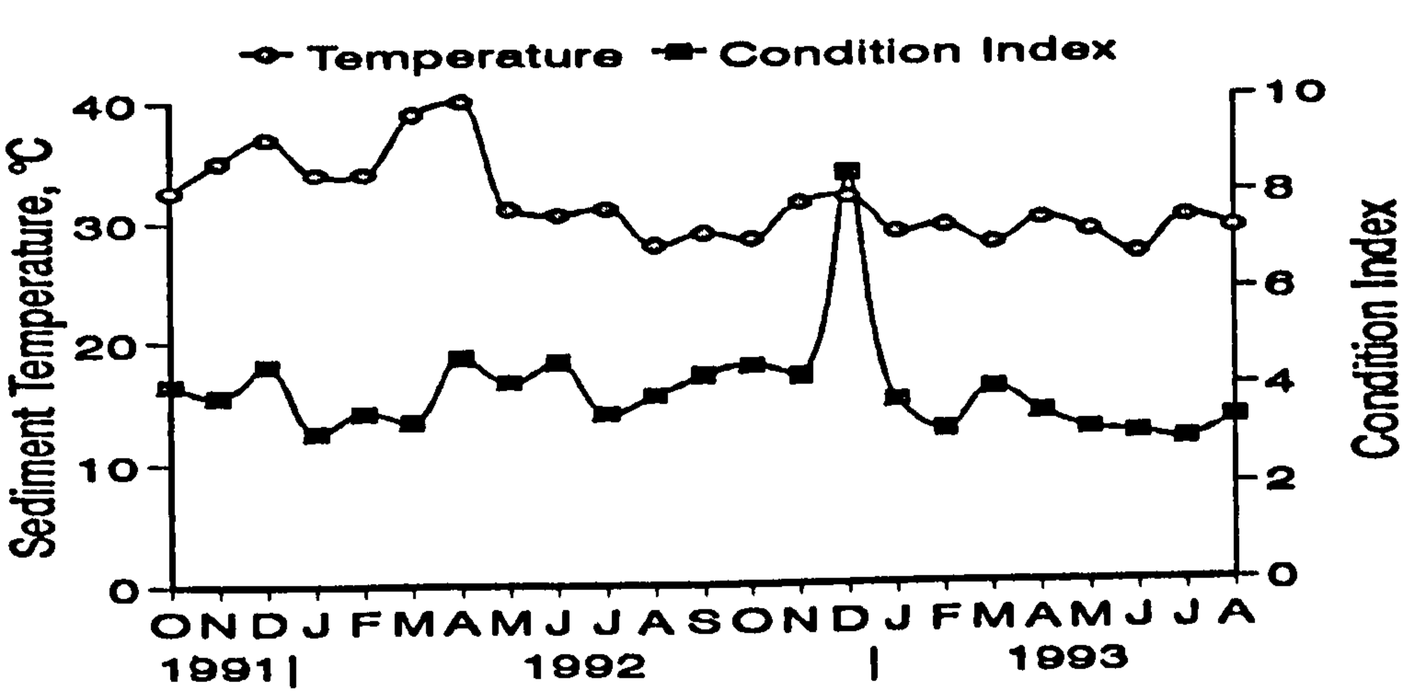
C


Gonad Index
Condition Index
Population Gonad Index
Condition Index
8
6
4
2
0
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

D


Gonad Index
Temperature
Sediment Temperature, ℃
Population Gonad Index
40
30
20
10
0
4
3
2
1
0
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

E


Temperature
Condition Index
Sediment Temperature, ℃
Condition Index
40
30
20
10
0
10
8
6
4
2
0
O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

a significant influence on the condition index of the clams ($F=12.35$, $P<0.001$, $df_{(1, 943)}= 6.63$, Table 13A); as does the interrelation between size and season ($F=12.08$, $P<0.001$, $df_{(1, 943)}= 6.63$, Table 13A). Here the result is that at year 1, starting from September 1991 until August 1992, samples collected at the onset of the rainy season, i.e. November and December 1991, showed a significant positive correlation compared with samples collected in May-June 1992 (dry season) which exhibit a negative correlation between these two parameters (Table 13, Figs. 9, 24 and 27A). In November and December 1991 when the clams were in the spawning stages, tissue weights were heavier than those collected in May-June 1992, i.e. when they were in the redeveloping stages. This results in these positive and negative correlations.

In year 2 from September 1992 to August 1993, it was again in September 1992 during the intermonsoonal period and in January 1993 when there was the highest monthly rainfall in Bandengan (Fig. 9), that the two associated parameters exhibited a very positive correlation ($P<0.001$, Table 13B) compared with May-June 1993 at the mid dry season ($P<0.001$, Table 13B) where there were a significantly negative correlation between condition index and size (Figs. 24 and 27A). At one point during rainy season (Fig. 24, October 1992) there was also a significantly negative correlation between condition index and size ($P<0.001$, Table 13B); but this was after a very complete spawning period particularly by the females. In those months when the condition of the clams increased with size (November-December 1991, September 1992 and January 1993) the oxygen saturation value as well as the chlorophyll-a concentration in sea water were high (Fig.9E). Nevertheless, Table 11 reveals that in general, both gonad and condition indices did not show any particular relationship to any of the physical parameters measured.

Only in the case of dissolved oxygen content and sediment temperature did the condition index show a relatively high but not significant positive correlation (r= 0.259 and 0.297 respectively, P>0.05) whilst gonad and condition indices as well as tissue weight maintained a negative correlation (r= -0.178 and -0.055 respectively, P>0.05) to chlorophyll-a concentration. Moreover, as for *A. granosa*, Figures 27C-D illustrate that the gonad index in *A. antiquata* exhibits a complex nonlinear relationship to condition index and to the sediment temperature, even though the trend is there. This was suggested since the values for the Spearman rank order of correlation are close to zero (r= 0.030 and 0.015, P>0.05).

It is interesting to observe how the regression lines in *A. antiquata* pivot from a positive to a negative correlation (Fig. 24). When the animals are ripe or spawning, the correlation between the two parameters remained positive (September 1991-March 1992) albeit with lower slopes. In April-June 1992, when the blood clams were redeveloping the correlations become negative. However, where there is an extended period of spawning, as in this species, it is difficult to assert whether this negative correlation was due to the spawners/adult population releasing reproductive material or because juvenile clams (20-24mm) were entering the population. It was assumed from the histological study that these juvenile individuals were still in the accelerating somatic growth, and not yet sexually mature, thus resulting in a relatively low condition index and therefore causing the regression lines to shift toward the negative correlation. In July-September 1992 when the majority of clams in the population were spawning, the slopes of the regression lines stay positive but lower. At the peak of the spawning period in October 1992, it shifted to become negative and in November 1992 when more individuals were re-

maturing (Fig. 16B) while others were completely spent, the regression swung back to a positive relationship.

Although the spread of size range in the December 1992 sample did not represent the juveniles, it can be seen that larger individuals (>48mm) caused the regression line to become significantly negative. Again in January-February 1993 when the population consisted mainly of ripening and spawning individuals, the correlation became positive. The slope was very steep as both sexes were ready to spawn (March 1993) then it gradually declined (April-May 1993) when the gonad was redeveloping. In June 1993 there was evidence of spawning and again it is not clear whether some recruits of 14-22mm make the slope negative or whether this is due to the spawning activities in the adult population. In July-August 1993 clams were ripening and the slopes again reverted into positive relationships.

3.4. Discussion

The stages of spermatogenesis and oogenesis for both *A. granosa* and *A. antiquata* are similar to the usual pattern described for bivalves, though no follicle (nutritive) cells of the sort observed in *Paphia staminea, Macoma balthica, Abra alba, Abra tenuis* or *Tapes philippinarum* (Quayle, 1943; Caddy, 1967; Nott, 1980; Sbrenna and Campioni, 1994) were observed in either sex of both species. More specifically, gametogenesis in these anadarinids is similar to that described for *Venus striatula* (Ansell, 1961) where the gametogenic cells located within profusely branched follicles are nourished by the surrounding mesenchyme or vesicular connective tissue.

The gametogenesis process within the lamellibranch testis has received little attention in the literature compared with that for the ovary. The descriptions by previous authors suggest that this process parallels the

classical vertebrate pattern, i.e. succsessive layers of spermatogenic cells (spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa) occurring more or less regularly in succession towards the centre of the follicle (Coe & Turner, 1938; Quayle, 1943). The account of Coe & Turner (1938) for *Mya arenaria* states that sperm are proliferated from spermatogonia on the follicle wall. Although no plates of the earlier stages are presented by any of these authors, the sequential arrangement of the germinal cells in male reproductive tissues of both species studied here appeared to be the same as their descriptions.

The blood clam *A. granosa* has separate sexes but is not sexually dimorphic (Pathansali & Soong, 1958; Pathansali, 1964; Broom, 1983; Wong et al, 1985). However, all these authors reported that the sexes appear to be in an approximately 1:1 ratio and no sex changes have been reported thus far. The results of the present study found that the overall ratio of males to females in Wedung is 1.49:1 but in Tapak it was 0.76:1 in favour of females. Apparently a protandric type of development occurred in Wedung and Tapak as there was evidence of a change in sex ratio with size. In both populations, the majority of 15-30mm clams are males. The sex ratio shifts to become 1:1 when the animals were between 30-40mm in length and by the time they attained a size over 45mm the populations were dominated by female individuals.

Approximately 96% of bivalves have been reported as being gonochoristic (Morton, 1958), the remaining 4% exhibit one or more of the following grades of hermaphroditism: 1) Simultaneous hermaphroditic (ambisexual) species in which: a) sperm and ova are formed in different region of the same gonad, e.g. most Pectinidae, some Tridacnidae and many members of the Unionidae, and b) a distinct ovary and testis open separately

for instance in many members of the order Anomalodesmata, 2) Sequentially hermaphroditic species have one sex change in their life history (or many, e.g. oyster) and are generally protandric, e.g. *Venus mercenaria* (Loosanoff, 1937) and *Astarte sulcata* (Saleuddin,1964).

Lucas (1975) showed that the percentage of hermaphrodites amongst juvenile bivalves may be quite high, for instance, 23% for *Venerupis decussata* (L.), 44% for *Venus striatula* (da Costa) and 72% for *Glycymeris glycymeris* (L.). These proportions are all relatively large and therefore important. However, it was not reported whether those hermaphrodite juveniles survived to become adults. Subsequently, Broom (1983b) found evidence of only 0.33% (1 out of 300 specimens, size not indicated) hermaphrodites amongst *A. granosa* in West Malaysia. In comparison to that, the figures of 1.43% and 1.45% for hermaphrodite individuals in the Wedung and Tapak populations were still within the range reported for other populations.

Narasimham (1988) in India found no hermaphrodite *A. granosa* individuals over 4200 specimens examined throughout a four years study (3840 were from smear and 360 from histological preparation) and that the sex ratio did not differ from 1:1. From the data presented by Narasimham (1988) it is obvious that for small mature individuals ranging from 22-28mm, the occurrence of male clams was much higher than females (3:1 or 99:33 individuals), and that 82.1% clams of 18-20mm were male as no female individuals were encountered. Furthermore, in accordance with the findings in the present study, males outnumbered females in the first 22-38mm groups, females dominated in the rest of the length groups (40-62mm) and at 70mm all were females. Yet, somehow the author calculated that the sex ratio was not different from 1:1.

Kastoro (1978) reported an occurrence of 1.2% hermaphrodite individuals with a male to female sex ratio of 1:1.4 in a population of *A. antiquata* in West Java, Indonesia. On the contrary, the male/female sex ratio recorded for *A. antiquata* in the present study is 1.47:1. Surprisingly, of the 1,040 of *A. antiquata* examined, Toral-Barza and Gomez (1985) in the Philippines did not find any hermaphrodites. They noted that there were more males than females although the sex ratio did not differ from 1:1. Similarly, Kayombo and Mainoya (1987) in Tanzania established that the ratio for *A. antiquata* did not depart from 1:1 (1:1.13, 253 individuals). Yet from one of their graphs (Figure 6 in Kayombo & Mainoya, 1987), it is obvious that over the size range 26-30mm the percentage of male individuals was >90% whilst the female was <10%. The proportions approached each other as the clams grew to a larger size and the ratio becomes exactly 1:1 amongst clams of 41-45mm shell length. These observations were remarkably similar to the findings of the present study.

Moreover, Kayombo and Mainoya (1987) have also found a higher percentage of hermaphrodites (i.e. 9.88% compared to only 0.84% in Bandengan). The examination of gonad smears misidentified these hermaphrodites as functional females, before detailed histological sections revealed that they were in fact hermaphrodites. Here, the hermaphrodites were macroscopically misidentified as male clams of 32-42mm with whitish coloured gonads, but the sections revealed that a set of oogonia develop simultaneously and the blood clams rapidly make a quick transition to females without the follicles being in regression. So, despite their small number which is insufficient to satisfy a hypothesis of occasional random sex change by many or all members of the population, these simultaneous

hermaphrodites in both *A. granosa* and *A. antiquata* could be interpreted as transitional phases of a protandrous sequential hermaphrodite.

Yoloye (1974) working on specimens of *A. senilis* from the Nigerian coast, reported that this species is a protandrous hermaphrodite and that all young specimens become functional males when only one month old, and, judging from the information given, 5-9mm in length. From the sixth month, many specimens appeared to be hermaphrodites and from the end of the first year the sex ratio had become 3.17:1 and remained more or less constant in the adult populations. By contrast, Yankson (1982) who studied Ghanaian populations of *A. senilis*, reported a completely different situation. Hermaphrodites occurred extremely rarely, i.e. 5 out of 1,448 and 3 out of 313 individuals in the two lagoon populations studied. There was no evidence of protandry nor, under the normal conditions of salinity, did the sex ratio deviate significantly from 1:1. However, in closed lagoons where, in the hot, dry season salinities rose to 50‰ and surface water temperatures were 32-34°C, the sex ratio did deviate significantly from 1:1, the actual observed ratio being close to 1:2 in favour of the females. Furthermore, primary gonads in this species begin to differentiate when the animals are 10-12mm long and the first spawning takes place at a length of approximately 20mm.

Little is known about the sex determining mechanism of bivalves and molluscs in general. So far it is known that there are no morphologically distinguishable sex chromosomes and it is thought that hormone balance determines whether an individual changes sex (A. Beaumont, pers. comm.). Recently, Allen & Guo (1994) suggested that the dwarf surf-clam *Mulinia lateralis* is more likely to have an XX and XY (like many vertebrates) than an XX:2A and XY:2A (as in the fruit fly *Drosophila spp.*) sex determining mechanisms. However, karyotypes have shown no evidence of heteromorphic

sex chromosomes. Nevertheless, development as a male is considered to require less energy than as a female (Calow,1983; Russell-Hunter & McMahon, 1975). For example, one germ cell will produce four sperm which largely consist of a nucleus surrounded by cytoplasm specialised for locomotion and penetration of the egg. In females, one germ cell will develop only to become one large oocyte with energy-rich yolk and cytoplasm. So, presumably the advantage of firstly being male is that some energy could be saved and redirected towards somatic growth because there is a trade-off between growth and reproduction (Calow, 1983; Seed & Brown, 1977b). This could perhaps explain the preponderance of juvenile males within the Wedung population.

Moreover, it will be seen in Chapter IV that the population in Wedung has a higher K value of von Bertalanffy growth equation, i.e. $0.78.y^{-1}$ compared to $0.34.y^{-1}$ in Tapak, indicating that *A. granosa* in Wedung attained their asymptotic size earlier than those in Tapak. The histological study indicated that although the population in Wedung may continue to reproduce throughout several seasons (iteroparity), clams tend to grow rapidly to small adult size and have a short pre-reproductive life span which is one of the characteristics of semelparous species (Calow, 1983). Environmental factors such as site location, finer grain size of the bottom sediment, food availability and relatively less competitor and predators (e.g. the small turret gastropod *Cerithidea cingulata* and brachyuran crabs) may be taken into account for an optimum growth in Wedung than in Tapak, although the majority of blood clams in Wedung cannot complete their second year of life.

Comparatively, the smallest specimens collected in Tapak were always larger than the smallest clams in Wedung, and were all sexually mature,

although only a very few of them were encountered. This sparsity of juveniles indicated that Tapak may be a less suitable habitat for this anadarinid species.

The population in Tapak however, has a relatively slower growth rate but enjoys enhanced longevity since these clams have never experienced any artificial pressure (human harvesting). There, the occurrence of male individuals differs from that in the Wedung population as indicated by the lower overall sex ratio (0.76:1). Whilst the rather sandy substrate seems likely to represent a sub-optimal habitat for this species, the total absence of juvenile males from the population helped to account for the very low density of blood clams in Tapak. However, it is not easy to explain the total absence of these juvenile males. Larger specimens of swimming crabs were more abundant in Tapak than in Wedung, as were wading birds and the presence of grouper and marine catfish, all of which have been described as potential predators of blood clams (Broom, 1983a, 1985; Pathansali, 1964). Moreover, Morton (1963) stated that since the reproductive rhythm in bivalves and molluscs in general is finely balanced, the determination of sex was so labile that nutritive conditions can tip it either way. Different mortality rates against the larvae and spat caused by factors other than food, which was also less abundant than in Wedung, could possibly explain the skewed sex ratios in Tapak. Furthermore, the sex change in already sexually differentiated individuals results in the higher proportion of females in the adult populations.

A. granosa usually mature in the first year by which time the clams have attained a length of 18-20mm (Pathansali & Soong, 1958; Narasimham, 1969, 1988). The smallest size at which gonad development has been observed was between 15-16mm (Pathansali, 1964) or 18-20mm for male and 24mm for

female (Narasimham, 1969, 1988). Their findings were in line with those obtained by Broom (1983b). Working in the same areas as Pathansali (1964), Broom (1983b) examined two size groups (15.7mm-17.5mm and 17.5-20mm) of *A. granosa* in September 1977 and found no evidence of the development of gonads although there was evidence that individuals in the upper part of the size range (17.5mm-20mm) had developing gonads by January 1978. Having identified the gonad changes in every three month with the September data as the only set examined for smaller individuals (15.7-17.5mm), Broom (1983b) confirmed that gonads did not develop until a length of 17.5mm had been attained and the first spawning occurred at a length of 24-25mm. A little earlier than Broom (1983), Yankson (1982) stated that in *A. senilis* the gonad begins to differentiate after the clams attain a shell length of 10mm. From 11mm-12mm onwards a majority have undergone sexual differentiation and can be identified either as male or female, and the author concluded that these clams did not begin to spawn until they are 20mm long.

In line with Pathansali (1964), except for a few precocious individuals of 10mm-12mm, gonads were distinguishable in animals of 15.6mm-15.7mm length in Wedung. Sections from juvenile clams of 15.6mm-17mm and 17.1mm-19.9mm in 1992 (February, June, September, October) and 1993 (April, June, August) revealed that the smaller size range as well as the larger clams were in a wide range of gonadal development. There was undoubtedly some spawning activity although only a few individuals were completely spent; 94% of these were males. This means that they had probably just started to spawn for the very first time or had spawned at least once. So, there was no evidence that first spawning has taken place quite some time after the onset of maturity, as observed in the populations of *A. granosa* in West

Malaysia (Pathansali and Soong, 1958; Broom, 1983b) or *A. senilis* in a Ghanaian lagoon West Africa (Yankson, 1982).

In *A. antiquata*, sections taken from 18.4mm-26.8mm juveniles at various times of the year (October 1991, February, June, September, October 1992, January, February, May and June 1993) showed that a majority of the population from 20.7mm onwards has undergone sexual differentiation and were in various stages of development; 65.2% has been identified as male and 34.78% as female. Comparatively, from a population of *A. antiquata* in Tanzania it was noted that size at first maturity was 26-27mm; these juvenile clams were also identified as male. The majority of clams from 28mm onwards had ripe gonads and the smallest females with ripe ovaries were 31-33mm shell length (Kayombo and Mainoya, 1987). Although the population size range of both the present study and that of Kayombo and Mainoya (1987) overlapped (14-58mm compare to 10-60mm), the result of both studies should nevertheless, be interpreted with care since no juveniles smaller than that size were examined.

A further comparison with the study in Philippines (Toral-Barza and Gomez, 1985) was not possible because these authors carried out their observation on clams of 38.5-50.7mm. They found that in 1978-1979 heavy spawning occurred practically throughout the year, with spawning peaks from May to April and from June to September. In the following year 1979-1980 intense spawning was apparent only between July and September. Fluctuations of the gonad index suggest that the duration of gametogenesis is 1-2 months. The values of the gonad index remained at ≥2.0 and indicated relatively synchronous gonad development and spawning in both sexes of *A. antiquata*. Moreover, the inactive stage where gonad follicles are small with few recognisable sex cells, were rarely found in the male reproductive cycle.

In agreement with their findings, in the present study the tendency for the gonad index to drop below 2.0 was observed more often in females than in males, even for *A. granosa.* That male individuals of both *A. antiquata* and *A. granosa* never reached a gonad index of zero was also confirmed by this study, but this does not mean that there was no fully spent stage in males as shown in Plates 5D-E. Thus, variation in the number of individuals spawning at any given time occurred during this two year study in Central Java.

For other congeneric species Squires et al (1975) observed that the smallest mature male specimens of *A. tuberculosa* in some Colombian populations were 32mm in length, whilst the smallest females were 36mm. The sexes were reported as gonochoristic but the sex ratio was not accurately determined. *A. trapezia* also has a sex ratio of 1:1 with no evidence of protandry, the gonads becoming macroscopically visible when the animals were 20mm long. Similarly, only *A. cornea* longer than 20mm have visible gonads (Broom, 1985).

The highest population density of *A. granosa* in Wedung recorded on August-September coincided with the seasonal appearance of 12-16mm juveniles. Assuming that most bivalve species release their gametes directly into the water, then such spawning might require an environmental trigger analogous to that provided in temperate areas by rising or falling temperature (Morton, 1963; Caddy, 1967; McMahon, 1983; Numaguchi, 1994; Sbrenna & Campioni, 1994). In this study, both gonad and condition indices of *A. granosa* are significantly and positively correlated with such physical parameters as sediment temperature, oxygen availability and phytopigment concentration reflected as chlorophyll-a. Indeed sediment temperature was the most prominent parameter influencing the reproductive activity of *A. granosa* in the present study.

The seasonal pattern of gonad index in Wedung and Tapak revealed three occasions in the rainy season when blood clams were ready to spawn and this coincides with the rise in sediment temperature and fall in salinity. Accordingly, Wong et al (1985) demonstrated that in the laboratory, spawning in *A. granosa* could be induced after repeated exposures to cold (17±1°C) followed by warm (34±1°C) sea water.

Some earlier investigations on *A. granosa* are confined to the time that spawning occurs as evidenced either by the time of the year that larvae appear in the plankton (Pathansali, 1964; Narasimham, 1969) or the time of the year that juveniles first appear in the bottom sediment (Nair, 1986). In Penang, Malaysia, the major spawning period of *A. granosa* is from July to October with a peak in August/September. A little further south, in Perak, Pathansali and Soong (1958) deduced, on the basis of the time of appearance of larvae, that it is a little later; and in Kuala Selangor, spawning takes place between September and November (Broom, 1983b).

The reason for this seasonality is uncertain, but seasonal salinity fluctuations in the Malacca Strait (Pathansali, 1964) are thought to play a major role. Broom (1982b, 1983b) has shown that at Kuala Selangor in 1977 there was a distinct depression of surface salinity in October/November and that this coincided with a major spawning. However, Broom (1983b) has argued that spawning is almost certainly linked in some way to seasonal salinity depression, although it is possible that the depression in salinity itself is not the factor that serves as the spawning cue. Furthermore, the period of high rainfall that may also depress temperatures on the intertidal mudflat is thought to be another possible environmental cue.

Conversely, in Wedung and Tapak, rainfall creates a warmer temperature and obviously decreases salinity, and it appears to trigger ripe

Table 14: Reported spawning time and/or recruitment of the anadarinids from tropical and temperate waters

Species	Locality	Spawning time	Authors
A. granosa	Penang, Malaysia	August-September	Pathansali & Soong, 1958
	Perak, Malaysia	August-September	Pathansali, 1964
	K. Selangor, Malaysia	September-November	Broom, 1983b
	Penang, Perak & Kuala Selangor, Malaysia	April-June & August-October (1979-1985)	Nair, 1986
	Kakinada Bay, India	January, April	Narasimham, 1969
	Phuket Bay, Thailand	October-November	Boonruang and Janekarn, 1983
	Kakinada Bay, India	varied, all-year round	Narasimham, 1988
	Central Java, Indonesia	August-November	Sudradjat, 1978
	West Java, Indonesia	May-June	Ismail, 1979
	Central Java, Indonesia	July-August	Present study
A. antiquata	West Java, Indonesia	August-December	Kastoro, 1978
	Calatagan, Philippines	July-September	Toral-Barza and Gomez, 1985
	Dar es Salaam, Tanzania	July-November	Kayombo & Mainoya, 1987
	Central Java, Indonesia	July-February	Present study
A. senilis	Lagos, Nigeria	October-November	Yoloye, 1974
	Sierra Leone	November-December	Okera, 1976
	Ghana	all-year round	Yankson, 1982
	Senegal	July-September	Debenay et al, 1994
A. subcrenata	Japan	July-August	Cahn, 1951; Ting et al, 1972
A. broughtoni	Japan	June-August	Kan-no & Kikuchi, 1962
	Korea	August-September	Yoo & Yoo, 1974
	Sea of Japan	July-September	Dyzuba & Maslennikova, 1982
A. granosa bisenensis	Japan	June-August	Cahn, 1951
A. ursus	Japan	July-August	Tanaka, 1959
A. trapezia	Australia	late summer	Sullivan, 1960

clams to spawn. Similarly, Boonruang and Janekarn (1983) found evidence of a spatfall at Phuket, Thailand, in October-November 1979 during the transition between the rainy and dry season. Narasimham (1969) also stated that *A. granosa* in Kakinada Bay, India, spawns throughout the year with two spawning peaks, one in January and the other in April. A more detailed study in the same area for the same species by the same author reported that there can be 2-4 reproductive cycles in a year and that their time varied considerably between years (Narasimham, 1988). It has been shown in this study that irrespective of sites, *A. granosa* spawns continuously unlike its congener *A. antiquata.*

The seasonal gonadal changes of the populations of *A. granosa* in Wedung and Tapak showed a low level of spawning activity throughout the year with a peak in July-September 1992, which in Tapak is distinguished by a wide range in the confidence interval in oocyte density. There was also conclusive evidence for a prominent spawning in October-December 1991, which is assumed as one of the minor spawning peaks occurring in the rainy season, as it was in November 1992. Despite the severe environmental conditions from October 1992 to March 1993, when the salinity in November 1992 was slightly increased but still below normal, i.e. from 13‰ to 17‰, and the temperature was at its highest - a combination which induced spawning, it was noted from the histological study that a minor spawning did occur in November 1992. Besides, there was some evidence of a major spawning in both August 1992 and 1993 in Wedung and to a lesser extent in Tapak, i.e. August-October 1992 and June-August 1993. The major spawning peak in June-August 1992 at Wedung is indeed less prominent than either the minor spawning peak in November 1991 or the major peak in June-August

1993, however it markedly reduced the shell growth within that three months period (see Fig. 36C in Chapter IV).

In agreement with the observations carried out for a population of *A. granosa* in Kakinada Bay, India, (Narasimham, 1988), the major and minor breeding season in the present study varied throughout the two year period but the pattern of development has remained the same. During the minor breeding period (November 1992), gonads of comparable fullness to the gonads in the major breeding season (August 1992) were redeveloped without follicle regression, whilst spawning was partial and incomplete.

In a series of laboratory experiments Wong et al (1985) reported that spawning in *A. granosa* began with the males. Examination of the gonads of individuals which had stopped discharging, revealed that a considerable amount of gonadal material remained in the follicles. It would appear that the magnitude of the minor spawning following the major one depends on the degree of gonad development and intensity of spawning during the major breeding season. Such observations are in accord with the view of Pathansali (1964) who assumed it to be related to the hydrographical conditions.

Indeed *A. antiquata* in Bandengan does not show any marked recruitment, but histological studies confirmed that it does have a major spawning in November-December as indeed does *A. granosa* in Tapak, though recruitment appeared to be negligible in the latter population. An inadequate sampling technique which did not include sieving the sediment excluded spat from the samples, whereas in Tapak, this was coupled with the fact that this is a site of potentially declining environmental water quality.

Seasonal fluctuation in breeding activity which is related to the rainfall pattern apparently is not only confined to *A. granosa*, since it has also been observed in various gastropods in peninsular Malaysia including intertidal

forms (Berry, 1975a, 1982, 1983). From Table 14, it can be summarised that populations of *A. granosa* from different localities may exhibit biennial spawning peaks, i.e. April-June and August-September, whereas for *A. antiquata* spawning was more restricted to the second half of the year, i.e. July-November.

In general and like other bivalve species, the condition index in both *A. granosa* and *A. antiquata* increases as the blood clams grow to a certain size. Beyond 50mm, the increase in condition index slows down. Previous studies on meat yield in bivalves including that of Nascimento et al (1980) reported that in the mangrove oyster *Crassostrea rhizophorae* condition index increases rapidly as the oysters grow from 20 to 60mm. The increase in meat yield however, declined as individuals grew from 60 to 70mm. The work of Nascimento and Pereira (1980) revealed a poor condition index in *Crassostrea rhizophorae* during the spawning and post-spawning periods respectively. Studies by Durve (1964) on C*rassostrea gryphoides* and Algarswamy (1966) on *Donax faba* revealed that the condition index of these bivalves fluctuated closely with the reproductive cycle. Those observations are in accord with the pattern in both species in the present study, condition index declining when the animals had just spawned or were in the early stages of redeveloping their reproductive tissue, and rising as the animals ripened and became ready to spawn.

Pinnotherids (pea crabs) are a group of brachyuran crabs adapted for life within other marine animals such as holothurians, annelids, ascidians, tunicates, echinoids and polychaetes (Ruppert & Barnes, 1994). As a group pinnotherids have a host list of at least 21 species, but mostly they thrive in the mantle cavity of bivalves (Lim, 1966; Seed, 1969; Morton & Morton,

1983; Bierbaum, 1985; Bierbaum & Ferson, 1986; Bierbaum & Shumway, 1988; Narasimham, 1988; Haines et al, 1994).

In the literature however, there is no consistency when defining the nature of the association between the pea crab and its host. Morton and Morton (1983) described the relationship as commensalism, or amensalism for male pea crabs and parasitism for females (Haines et al, 1994). At least, to some extent the pea crab benefits at the expense of its host (Bierbaum, 1985; Bierbaum & Shumway, 1988) although it was reported not to cause any damage to the host's tissue (Bierbaum & Ferson, 1986; Haines et al, 1994).

Overall in this study, 241 pea crabs were found in the 5254 clams colleted from Bandengan (4.59%). This was comparably lower than the degree of occupancy found by Haines et al (1994) in *M. edulis* from two natural populations in the Solent, UK, where 2481 individuals out of 5366 of the specimens studied (46.24%) were inhabited by one or more pea crabs (*Pinnotheres pisum*). Moreover, they found that 5.74% of the mussels studied were occupied by a pair of male and female pea crabs. This multiple occupancy was never found in *A. antiquata,* perhaps because the average size of the clams (30-34 mm) is smaller than that of the mussels (44.61-49.15 mm) although the average size of the pea crab is remarkably similar, i.e. 4.38 to 4.48mm for males and 7.82 to 8.13mm for the females in the Solent compared to 5mm and 7mm for males and females respectively in Bandengan. Thus living space in the clams will be overly constrained to accomodate more than one crab.

Results in this study suggest that female pea crabs in particular are parasitic. Crab-infested clams have poorer physiological condition than non-

infested clams because these have a significantly less tissue weight than non-infested clams of comparable size ($F= 6.37$, $n= 481$, $0.012<P<0.05$).

In the present study there was only a single occurrence of a pinnotherid crab within the mantle cavity of *A. granosa* at Wedung and none were recorded at Tapak. Narasimham (1988) studied a population of *A. granosa* in Kakinada Bay, India, and found that a pea crab *Pinnotheres alcocki* occupied ca. 10.8% of the population. The range of occupation varied from 0-46% within different months of the year and similarly, multiple occupation was uncommon (2%). Although the crab was reported not to cause any damage to the organs of the host, the condition index was similarly low as observed in *A. antiquata*, i.e. in 41.1% of the crab-infested clams compared to those of uninfested clams.

Reproductive and physiological condition (the latter is presented as both condition index and dry tissue weight) of both *Anadara* studied showed consistently negative correlations with respect to salinity (Table 11). This finding may support the conclusion that they are typically true estuarine organisms with marine affinities which live in the central parts of estuaries. According to McLusky (1989) most true estuarine organisms could live in the sea but apparently their absence from the sea is due to competition from other animals. This hypothesis of spatial dispersion for *Anadara granosa* and *A. antiquata* should probably be tested in the future, because some other species of anadarinids do occur in the sea, e.g. *Scapharca cornea*, *Cunearca pilula*, *A.* cf *ehrenbergi* and a species identified by J.D. Taylor (pers. comm.) as *A. uropygimelana* from Hong Kong waters.

The reproductive activity for *A. granosa* in Wedung is significantly and positively correlated with sediment temperature, chlorophyll-a concentration and oxygen availability, whereas the population of *A. granosa* in Tapak

showed non-significant negative correlations to almost all of the related parameters (Table 11).

A detailed study regarding the diet of *A. antiquata* in its natural habitat revealed that this species is strictly phytophagous (Kasigwa and Mahika, 1991) for no zooplankton were found in the digestive system of the specimens examined. In the natural environment, peak reproductive activity is usually linked to the peak of food abundance or these may overlap in order to assure that plenty of food is available for the offspring. The population gonad index of *A. antiquata* in this study was negatively correlated ($r= -0.178$, $P>0.05$) to chlorophyll-a concentration, yet the sections regularly showed that the gut was full of phytoplankton and zooplankton remnants.

More recently, a model simulation for oysters suggests that particulate organic matter (POM) greatly overestimated food supply (the oysters accumulate biomass at an unrealistic rate), whilst chlorophyll-a tended to underestimate the food supply (oyster do not accrue biomass fast enough nor grow to sizes observed in the field), but a value called LCP (which relates chlorophyll concentration to the energy content of the seston measured as the sum of the caloric content of particulate lipid, carbohydrate and protein) closely tracked the oyster production (Soniat et al, 1994). It is thereby difficult to state what environmental factors trigger such activities for *A. antiquata* and *A. granosa* in Tapak, for these factors are interdependent It is thus concluded that the relationship between reproductive and general condition of *A. antiquata* to food availability and other environmental factors is not as simple as that for *A. granosa* in Wedung.

The information gained on the reproduction of these two *Anadara* species may be summarised as follows. Both *A. granosa* and *A. antiquata* are monomorphic gonochoristic species. Their planktotrophic type of post-

fertilisation development is associated with the production of large number of small eggs (45-65μm) during their iteroparous life cycle.

Amongst the small juveniles and young adults *A. granosa* of 18-30mm shell length, males outnumbered females thus resulting in the sex ratio which significantly departs from 1:1. The modal length of the overall populations (30-35mm) in this study coincides with the 1:1 sex ratio. This is the ratio usually reported in previous studies which then conclude that sex is stable in this species. However, amongst size ranges greater than the mode (>35mm), the sex ratio again departs significantly from 1:1, but now in favour of the females. Although less information was obtained for *A. antiquata*, this congener appears to have a similar type of development to *A. granosa*. The sex ratio in this species also departs significantly from 1:1. This difference appears to derive from the fact that *A. antiquata* grows to a larger body size prior to directing energy towards reproduction.

For *A. granosa*, particularly those from the Wedung population, high adult mortality due to harvesting may have resulted in a different reproductive strategy. Moreover, it appears that the time delay occurs between the first appearance of reproductive tissue and the commencement of spawning is not too long. Here, size at first maturity is somewhat smaller than previously reported in other studies. This smaller size at maturity however, may also be because Wedung offers such environmental conditions suitable for *A. granosa* as the site is situated in a flat and sheltered bay, protected from strong wave actions but with large fresh water influence, and with fine nature of the bottom sediment; besides it provides more abundant food and fewer competitors and predators. Nevertheless, more precise information is needed particularly since a thorough comparison with the adjacent natural population in Tapak, which is relatively undisturbed and

commercially unexploited, is impossible at this stage of the study. This is due to the declining quality of population in Tapak for which very few specimens are available and the fact that the population is dominated by large female individuals.

Although occurring in relatively low percentages, hermaphrodites are observed and a sequential type of protandric hermaphroditism is proposed, based on male dominance in the smaller individuals followed by female dominance amongst larger individuals.

The low level of spawning throughout the year with one seasonal spawning peak superimposed with minor spawnings which has been reported in this study broadly agrees with previous studies of *A. granosa.* Although these spawning peaks may vary considerably from year to year as well as from one population to another, it is clear in this study that February-March was the period when majority of the clams were developing their gametes, whereas June-September was the major spawning season. Since spatfall is quite seasonal in its occurrence, it is not therefore consistent with the low level of spawning activity throughout the year; this too broadly agrees with previous records. The peak of the spawning season in this two years study occurred during the dry season when salinity was at its highest. At that time of the season, temperature, oxygen saturation values, and chlorophyll-a were amongst the lowest. However, subsequent warmer temperature, high oxygen content combined with low salinity during months of rainy season when the food is abundant, also coincided with minor spawning periods in *A. granosa.* For *A. antiquata* it seems that the relationship between such intrinsic factors (reproductive investment, parental growth and survivorship) and extrinsic variables (e.g. temperature, salinity and food supply or oxygen availability) is

more complex. A more detailed and comprehensive study is needed to explain these relationships.

CHAPTER IV

G R O W T H

4.1. Introduction

The shells of the arkshells, *A. granosa* and *A. antiquata*, are composed of three distinct parts, i.e two laterally compressed calcareous valves which are attached and articulate dorsally by a non-calcified conchiolin (proteinaceous) in origin, elastic hinge ligament which is covered above by the periostracum (Ruppert & Barnes, 1994). Within the family Arcidae, there are a series of taxodont teeth along the hinge, the number and arrangement of which are important taxonomic features (Oliver, 1992). The teeth also function to prevent rotational and shearing movement of one valve over the other. The taxodont condition found in the Nuculacea and Arcacea is probably the simplest and most primitive type of hinge (Seed, 1980) which may have become reduced or be even absent in other taxa. The shell of the Arcidae is composed primarily of aragonitic calcium carbonate. A vertical section through a valve reveals three shell layers, i.e. an outer periostracum, an outer crossed-lamellar layer which is also found in the hinge and taxodont teeth, and an inner complex crossed-lamellar layer which is bounded by the trace of the pallial myostracum (Taylor et al, 1969).

The primitive taxodont condition has persisted in *A. granosa* to the present day with little or no change to most of their morphological attributes at least since the Pliocene period some 5.2 to 1.64 million years ago (Afiati, 1977; Harland et al, 1989). *A. granosa* and ark shells in general thrive in environments that are generally considered to be unsuitable to other bivalve species, and this has led one to consider that their physiology as well as their shell shape must have been evolved to aid in their survival (Afiati, 1977).

Growth can be measured as an increase in total biomass or volume. In bivalve molluscs, however, growth is more conveniently expressed as an increase in the linear measurement of the shell which contains a record of growth, either in the form of rings or as a series of fine numeric bands within the shell structure. However, on some occasions whilst an individual organism may be increasing in biomass, its growth in terms of other linear parameters may vary seasonally with age and in response to changes in environmental conditions. Wet flesh weight, for example, in *A. granosa* can vary enormously (Broom, 1982c) with the animal actually experiencing negative growth depending on the prevailing environmental conditions or the reproductive cycle or both. Loss in body weight may occur even though the shell continues to be deposited (Seed, 1980).

In recent years much has been learned about the relationship between growth of the shell and body weight changes (Bayne, 1976). The shell valves increase in size primarily by incremental accretion of calcium carbonate at the valve margins (Richardson, 1993; Ruppert & Barnes, 1994) and once deposited the gross morphology cannot be changed. The shells therefore provided records about the age and the growth history of the organism including the environmental conditions underwhich shell deposition took place. Conventionally, growth in bivalves is assessed in either one or two ways. Firstly, by measuring the rate of growth of one parameter relative to that of another or to the whole body, i.e. ***allometric growth*** and secondly relating the size of the organisms to their age which is called ***absolute growth*** (Seed, 1980).

Several methods have been used to estimate the absolute growth of *A. granosa* through: 1) the modal progression in size frequency distributions (Narasimham, 1969, 1988) 2) incorporation of absolute growth data into the von Bertalanffy growth equation (Pathansali, 1964; Broom, 1982c, 1983c;

Ng, 1986; Narasimham, 1988) or 3) measuring the distance between consequtive growth increments observed in shell sections (Richardson, 1987). No work has been undertaken to study the allometric growth in this species. Nor have there been any recent studies on the absolute or allometric growth of a related species *A. antiquata*, except an analysis of the modal progression in length frequency distributions carried out by Kayombo and Mainoya (1987).

4.1.1. Allometric growth

The concept of allometry was first proposed by Huxley and Teissier (1936 cited in Seed, 1980). One of the most significant effects of growth follows from the simple geometrical principle that while the area of a body increases by the square of its linear dimensions, volume increases by its cube. Thus, if geometrical similarity is maintained throughout growth, the area/volume ratios will progressively decline. However, because most changes between the external environment and the organism occur across body surfaces, relatively constant area/volume ratios are an adaptive necessity which can only be maintained by changes in shell shape (Seed, 1980). The study of the different ratios of growth between two parts of the body or between one part of the body and the whole organism is termed ***allometry***.

In view of its accretionary mode of growth, i.e.: by marginal increments, the bivalve shell has generally been considered in terms of a logarithmic spiral. Theoretically any increase in size may occur without any correspondent change in shape. In reality, this is rarely accomplished although it is approached by most bivalves before they reach their post larval stage. Most bivalves exhibit at least some degree of ontogenetic change during their shell growth (Seed, 1980; 1982). As body shape does not always

change uniformly with an absolute increase in the size of the whole animal as a result of different growth vectors operating at different points around the mantle edge, most allometric studies of bivalve shell shape therefore, have used bivariate data in which only two linear parameters (e.g. length, height or width) are considered at any one time.Such analyses could perhaps be open to critisism because bivalves also change their shape as they grow. Although such data will not satisfactorily describe changes in body form on their own, they do nonetheless allow simple and useful comparisons to be made of the variability in shell proportions among different bivalve populations. Such comparisons therefore, provide a valuable insight into the relationships between shell shape and environmental change in space or time.

The relationship between any two parts of the body (x and y) can be expressed by the following power function:

$$y = Ax^b$$

where A and b are constants. The exponent b, or coefficient of allometry, represents as an index of relative growth rate of the two variables compared; whilst A is the value of y when x is unity. Expressed in its logarithmically transformed form, the equation becomes:

$$\log y = \log A + b\log x$$

The slope (b) and intercept (A) of such transformed data can be estimated by simple regression analysis (Brown et al, 1976; Anwar et al, 1990; Tsontos, 1991). The slope is then compared to the isometry coefficient (β), a theoretical entity defined as the ratio of any dimension of the measured parameters. If b is not significantly different from β then growth is isometric, i.e. relative growth along the two axes compared is identical and thus geometric similarity is maintained with increasing body size. Allometry results when parameters change over time in different proportions and can

either be negative ($b<\beta$) or positive ($b>\beta$) in direction. If the dimensions of x and y differ, then different criteria of allometry will apply. For example, where y is weight or volume cavity of the shell (L^3 or cubical) and x is length (L, linear), then b=3 corresponds to isometry.

The determination of allometric relationships however, also possess somewhat of a statistical problem as neither of the compared size dimensions are truly independent, a prerequisite for the conventional application of linear regression models. However, Brown et al (1976) have shown that the application of geometric mean regression alternatives provide allometric relationships not significantly different from those calculated conventionally when the two variables compared are closely related as in the case of shell/tissue measurements. Allometric regressions in the current study have therefore, been calculated in the conventional manner in accordance with the recommendations of Brown et al (1976) and protocols adopted in the current literature.

4.1.2. Absolute growth

4.1.2.1. Determination of growth from morphometric analysis

Demographic records of environmental change are based on the response of populations to temporal and spatial gradients in environmental quality and ecological stress. Such changes may be measured as changes in growth rate, recruitment, density, or survivorship as estimated from size or age-frequency distributions of skeletal growth. A single collection from a population will reflect the instantaneous structure of that population. By making several such censuses, spaced over at least one year, population recruitment, growth and mortality can be assessed.

By interpreting the modes in a polymodal size frequency distribution of *Zoarces viviparus* as distinct age classes, Petersen laid the foundations for the

study of age from population size structure (see Schnute and Fournier, 1980). Petersen's method is the simplest technique of determining the number and typical size of age classes present, but the application requires that the size overlap between adjacent cohorts is small. MacDonald and Pitcher (1979) demonstrated that polymodality constitutes an unreliable guide to population age structure, since the appearance of modes is dependent on a combination of distances between age classes means, size variance around these, the proportion of the population that each class represent, and the overall sample size.

Graphical length frequency analyses such as the probability plot methods of Harding (1949) and Cassie (1954) were an improvement on Petersen's modal analysis, in that a clearly defined protocol based on properties of Gaussian distributions was adopted for the resolution of component age distributions and their complete characterization in terms of mean, standard deviation and proportion statistics. However, subjective decisions undertaken in these analyses limit the reproducibility of results, particularly when size distributions are not polymodal (MacDonald and Pitcher, 1979). More recently Grant (1989) has shown that consecutive components must be separated by a minimum distance of 2.5 Standard Deviations for these graphical methods to be of any use at all, particularly if the sample size is small, and components are of unequal strength.

The size frequency distribution however, is a popular technique, which stems primarily from the longstanding view that it adequately describes the growth of bivalves and fish in particular (Cerrato, 1980; Schnute and Fournier, 1980). Its continued appearance in the literature has further reinforced its application by allowing comparisons between studies. Size frequency analysis for example is an important tool for studying the age of organisms such as crustaceans where suitable routine age determination

methodologies have not yet been established. Although for this group long term expensive tagging programmes can provide meaningful data. This kind of analysis allows large numbers of collected animals to be measured and then released back into the environment. Furthermore, size is the basis of many models of both ecological and fisheries related processes, e.g. predation, marketability, conservation, gear selection and acoustic target strength.

The utility of population attributes in deducing environmental change is related to how specific the environmental effect is on a population, as well as of the degree of resolution afforded by size-frequency histograms (Cerrato, 1980). Size frequency data can be analysed using the method of Bhattacharya which is another graphical method designed to disssect and describe mixtures of normal distributions (Bhattacharya, 1969). Grant (1989) has demonstrated that, although subject to the same fundamental limitations as the Harding/Cassie methods, the Bhattacharya method generally provides the most accurate results.

4.1.2.2. Determination of growth from microgrowth patterns

The absolute growth of individual animals may be described empirically in terms of the average size attained at specific ages, for which the rate of population growth can be approached. However, growth in bivalves is known to vary enormously, even individuals of initially similar size or age, or both, grown under apparently identical conditions can show considerable variations in their growth rates and this can be related to their genotype (Seed, 1980). Since it has been described that size on its own may not always be a good guide to the age of an individual organism, it is therefore necessary to back-up/strengthen the morphometric data by determining the age of individual specimens within the population.

One such technique originally developed for geological purposes, i.e. the analysis of growth patterns within the shell structure has proved to be a valuable method for determining the age and growth rate of many bivalves, especially marine species (Richardson et al, 1979; Lutz & Rhoads, 1980; Richardson, 1987; Anwar et al, 1990a; Ramon & Richardson, 1992; Richardson et al, 1990, 1993; Richardson, 1993; Tanabe & Oba, 1988).

By studying the pattern of spacing of microgrowth patterns visible in acetate peel replicas of polished and suitably etched shell sections, the growth rate of individual specimens may be estimated from different parts of the shell. The region of the shells where growth rate is usually assessed are the outer prismatic or crossed lamellar layer of the shell (Evans, 1972; Tanabe & Oba, 1988; Anwar et al, 1990a; Richardson, 1993), or the inner nacreous layer along the shell (Lutz & Rhoads, 1977), or from the umbonal region (Richardson et al, 1990), or a combination of several layers (Anwar, 1989).

Disturbance bands, umbonal shell abrasion and convergence of bands at the shell margin of older bivalves, together with factors affecting the clarity of the bands such as zonal position of the organism in relation to tidal immersion and the latitudinal origin of shells may complicate the interpretation of the growth patterns. However, the technique is generally accurate in distinguishing disturbance bands from those of truly annual origin and is invaluable in resolving growth over short time scales, e.g. tides or days (Richardson et al, 1980) and for studying the effect of environmental stressors on shell growth (Thompson & Richardson, 1993). Furthermore, the shell provides an ontogenetic record of growth and therefore can be used to provide a detailed record of the environmental conditions underwhich the organism was growing.

The acetate peel technique is a simple and inexpensive method. This method contrasts with an expensive yet recently developed technique which measures the variation in ^{18}O:^{16}O ratios in mineralising shell. The technique involves drilling calcium carbonate samples at intervals along the axis of shell growth. After suitable preparation and analysis in a mass spectrophotometer the samples have been shown to reveal seasonal variations in the $^{18}O/^{16}O$ which can be related to water temperatures and annual cycles of growth and hence age of the individual (see Krantz et al, 1984; Tanabe & Oba, 1988). Even though this oxygen isotope technique provides independent, objective and accurate results, the method does not lend itself to routine assessments with large numbers of individuals due to the considerable time and expense involved.

4.1.2.3. The von Bertalanffy growth function

Growth curves for both length and weight of many organisms are sigmoidal, and molluscs in general are no exception (Broom, 1982b; Anwar et al, 1990). Whatever curve fitting methods are used, growth is generally fitted to an equation of the von Bertalanffy or Gompertz type (Seed & Richardson, 1990). These quantitative expressions of growth allow useful comparisons to be made amongst different populations. The von Bertalanffy growth function is a three parameter asymptotic relationship widely applied in fisheries biology to model annual growth of exploited populations (Ricker, 1975). More recently, this relationship has been liberally extended to describe variations in short-term growth of bivalves (Richardson et al, 1980).

In the von Bertalanffy equation:

$$L_t = L\infty\ [1-e^{-K(t-t_0)}]$$

where L_t represents shell length of an animal at time t; $L\infty$ is the maximum asymptotic shell length; K is the growth constant reflecting the rate at which

maximum size is approached and t_o is a constant representing theoretical time when $L_t = 0$ (von Bertalanffy, 1938; Seed, 1980; Tanabe & Oba, 1988).

The Gompertz equation is similar but utilises the logarithm of length, i.e.:

$$\log_{10} L_t = \log_{10} L\infty \, [1-e^{-K'(t-t_i)}]$$

where K' is the rate of constant and t_i a constant representing time when L= 1 (Seed & Richardson, 1990). Thiesen (1973) used both equations for describing the growth of *Mytilus edulis* and concluded that the von Bertalanffy equation, which provides a more realistic estimate of asymptotic length, gave a good fit for individuals above one third of their maximum size. Meanwhile the sigmoidal Gompertz equation was more appropriate for smaller mussels. Bayne and Worrall (1980) have however, utilised the Gompertz equation in fitting growth curves to data on *Mytilus edulis*.

The von Bertalanffy and Gompertz equations assume that growth is determinate, i.e. that some maximum attainable size exists for any given population. Yet, growth in many bivalves may not always be determinate and therefore may not cease at any fixed adult size (Seed, 1980; Anwar et al, 1990). For this reason polynomial equations have been used to describe shell growth in preference to the more commonly used growth equations (Richardson, 1987).

Furthermore, the von Bertalanffy growth model cannot be applied to populations which are still in the exponential phase of growth (Anwar et al, 1990). Nonetheless, although the von Bertalanffy and Gompertz equations have been criticised, their use in curve fitting is perfectly acceptable provided that:1) there is some evidence of asymptotic growth and 2) it is appreciated that some uncertainty is always associated with these estimates (Seed, 1980). In applying the von Bertalanffy equation to data obtained from the progression of modal size classes in size frequency distributions of the blood

clam *A. granosa,* Broom (1982c) assumed that this model held only for sizes greater than 5mm. In the current study, the von Bertalanffy growth model will be applied to growth data obtained from the measurements of the microgrowth bands of several large specimens collected from the monthly samples.

As described previously in Chapter II, there are two ecomorphs of *A. granosa*, a rounded and an elongated form which were found at the study sites. One of the objectives of the present study is a) to compare the shell morphometry of the rounded form of *A. granosa* from the two natural populations (Wedung and Tapak), b) to distinguish whether there are any differences in the dimensions between the rounded and the elongated form of *A. granosa*, and c) to compare the morphology of the two morphs of *Anadara* which inhabit soft sediments and are thought to be more conservative in their shell shape than the byssally attached form (Seed, 1980) which is represented by *A. antiquata.* Seed (1976) concludes that probably the most reliable estimates of growth have been those obtained using a combination of methods. Therefore, in this chapter the periodicity of the microgrowth patterns, rate of growth and age of individual specimens is assessed.

4.2. Materials and Methods

4.2.1. Treatment of samples of blood clams for demographic and allometric studies

All clams were transferred into a large white collecting tray, from which 50 individuals covering the entire size range were selected at random for the allometric studies. Selected subsamples for the allometric study were rinsed in running tap water to remove any attached sediment and placed in boiling water for about 20 min until the valves opened slightly. The tissues for the allometric study were thus easily removed and allowed to drain on paper

Figure 28: Schematic diagram of the shell of *A. granosa* and *A. antiquata*

A. Shell valve of *A. antiquata* to show dimensions of the shell measured:

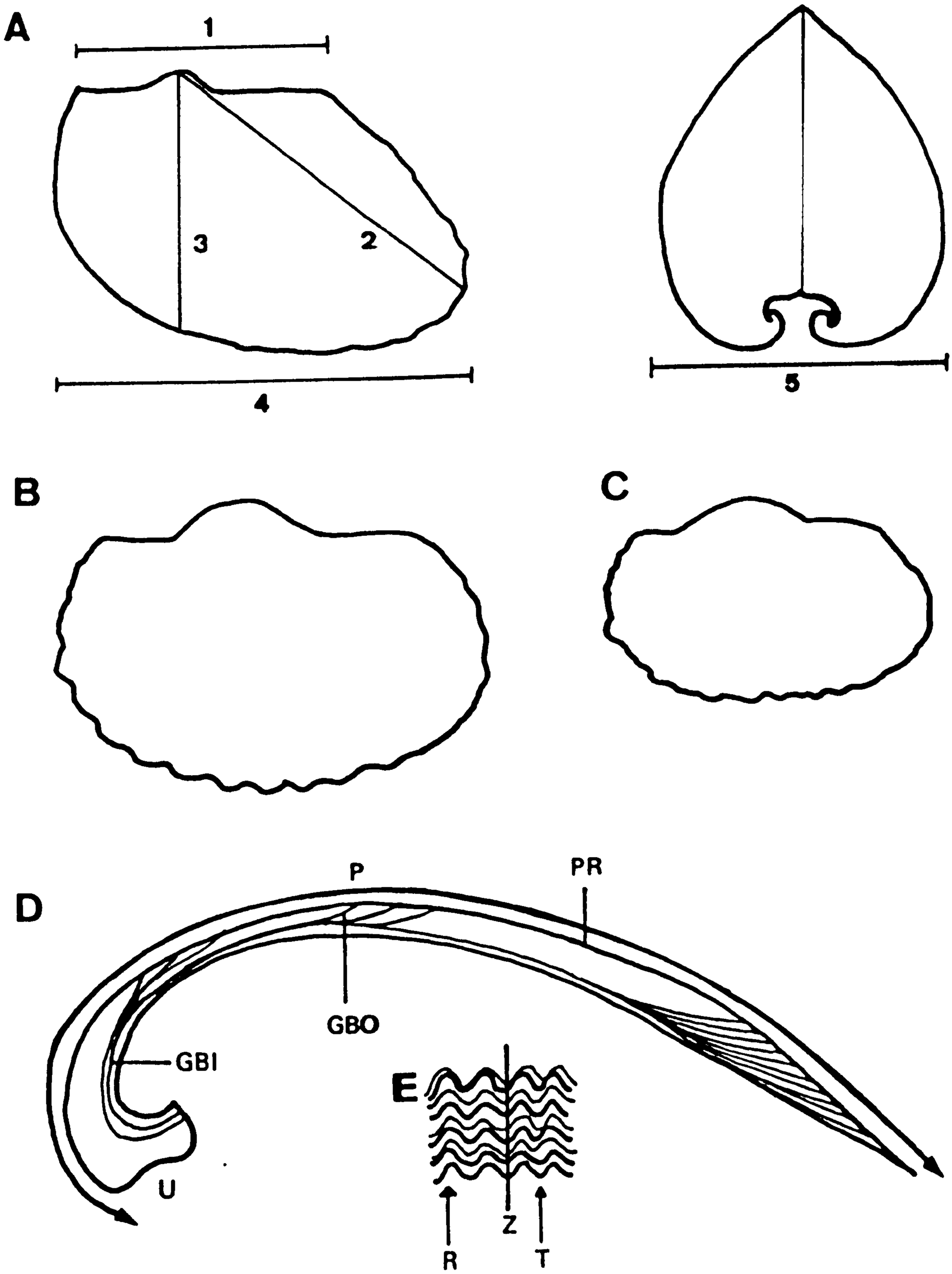
1. length of the hinge plate
2. height 1 or axis of maximum height
3. height 2 or axis of normal height
4. shell length
5. shell width

B. Shell valve of the rounded form of *A. granosa*. Detail of measurements in Figure 28A.

C. Shell valve of the elongated form of *A. granosa*. Detail of measurements in Figure 28A.

D. Radial section passing a trough at the axis of normal shell height, U umbo, GBI growth bands in the inner complex crossed lamellar layer, GBO growth bands in the outer complex crossed lamellar layer, p perimeter/surface of maximum growth. PR periostracum. For clarity only a few increments are shown.

E. Portion of a shell to show position of the section in Figure 28D. R ridge, T trough, Z is the axis of radial section.


A
1
3
2
4
5
B
C
D
P
PR
GBO
GBI
U
E
Z
R
T

towels before being placed into pre-labelled and pre-weighed plastic containers and set aside to dry in an oven at 60°C for 48 hours; the accompanying shells were left to dry at room temperature. Dry tissues together with both the corresponding shell valves were weighed separately on a Mettler balance to the nearest 0.0001 g.

Similar treatment in washing, boiling and drying the shells was carried out for blood clams obtained from the experimental plots, but particular attention was paid more to their shells. The numbered shells were wrapped individually and kept in pre-labelled plastics bag for subsequent shell sections. These samples together with some selected shells from the monthly sample specimens were then used to study the periodicity and seasonal variation in microgrowth increments within the shell structure.

The terminology employed for linear measurements of Queen scallop shell, *Chlamys opercularis*, by Taylor and Venn (1978) was used in this study. Shell length is the anterior-posterior axis of the clam with the hinge plate positioned parallel to the axis of the vernier calliper. Two measurements of shell height were taken. Height 1 is the maximal dorsal-ventral plane along the shell, measured from the umbo to the 'siphonal' opening at the rim, whereas height 2 is the dorsal-ventral dimension perpendicular to the hinge plate (Fig. 28). The greatest lateral axis perpendicular to the plane of the commisure was considered as shell width. The length of the hinge plate was also measured. All measurements were made to the nearest 0.01 mm using a dial vernier calliper.

4.2.2. Allometric growth

Shell dimensions, i.e. length, heights 1 and 2, width, hinge plate and weight of 50-77 individual clams were measured from each monthly sample. The data were logarithmically transformed and the allometric

relationships between pairs of variables were determined. Significant departure from isometric growth was assessed by a t-test, i.e.:

$$t_{obs(n-1 df)} = \frac{(b-\beta)}{\text{Std Error of } b}$$

Analysis of covariance using a single covariate (General Linear Model, GLM) was applied in a between-site monthly and between-form comparison. This analysis was carried out using data where there was a comparable size distribution of clams. Wherever possible shell length was consistently used as the independent variable. This dimension is the easiest to obtain, hence the most confident and accurately measured.

4.2.3. Absolute growth

4.2.3.1. Length frequency distributions

Considering that in the future both of the species studied, *A. granosa* and *A. antiquata*, may well become an important commodity in Indonesian fisheries, it was decided to examine whether an analysis of size frequency distributions would provide information on the age and growth of *Anadara*, such a methodology could then become incorporated in routine fishery practice. Population growth of both species has been assessed here using the method of Bhattacharya which resolves size frequency data into age classes.

For an analysis of the size frequency distribution of the population, the length data of the sample were grouped into 2 mm intervals, and the percentage frequency of each size class calculated and plotted into monthly length-frequency histograms. This analysis was performed using data from the monthly samples of *A. granosa* from Wedung and *A. antiquata* from Bandengan, but only carried out using data collected every four months from the *A. granosa* population from Tapak. Following estimation of the modal

sizes in the size frequency distributions, the results were plotted to construct growth curves for clams from the three populations.

A slight modification from the original method of Bhattacharya (1969), incorporating such features as the χ^2 and separation index are considered to lend greater credibility to the analysis (Tsontos, 1991), and these have recently been included in the Modal Class Progression Analysis (MPA) module of the ELEFAN v1.1 software package (Electronic Length Frequency Analysis; Gayanilo et al, 1988). The module allows dissection of age class component distributions and estimation of associated statistical parameters by Bhattacharya's method. Once identified, they were attributed to different cohorts of the population. Details of the module are included in Appendix 3.

4.2.3.2. Internal microgrowth patterns

4.2.3.2.1. Shell sections

Materials used for shell sections were obtained from blood clams grown experimentally at the three sites together with selected shells from the monthly samples. Similar techniques to those described in Anwar et al (1990) were used. The shells were first washed with mild detergent and warm water. Particular attention being paid not to damage the ventral growing margin of the shells. Once dried at room temperature and coded using a waterproof drawing ink, individual shell valves were embedded in resin to prevent fracturing of the shell during the process of sectioning. Metaserv resin type SW ('Slow') from Buehler UK Ltd. was used for this purpose. The procedure normally taking 10-15 hours for the mixture to set.

The small embedded shells were sectioned along the anterior-posterior axis, using a junior hacksaw or by means of a diamond saw for the larger specimens. The blocks were sawn in such a way that the section passed through a trough rather than a ridge on the shell surface (Fig. 28E), because

the trough contained no tubercles so its outer surface is regular and relatively well protected against abrasion during the burrowing process. However, because of the nature of the shell which, is slightly curved in an open spiral toward the posterior margin where the siphon-like mantle fusions occur, it was not practicable to make one section that passed through both the umbo and a trough at the vertical axis on the posterior end of the shell.

A preliminary investigation of the shell structure revealed that the outer complex crossed lamellar layer and clear growth bands at the growing margin of the shell (GBO in Fig. 28E) contained more information about the growth of the shell than the shell structure of the umbones. Therefore, all the sections were made so that they passed through the shell margins rather than through the umbones.

Halves of blocks resulting from radial cross-sections were ground initially on silicon carbide paper no. 400A and then on a glass plate covered with wetted aluminium oxide powder (Optical grade 50) until smooth. After such a sequential grinding, surfaces were polished using a polishing duster fitted into a circular frame and soaked with Brasso (household metal polish). After washing in a mild liquid detergent, taking care not to scratch the polished surfaces, the blocks were then etched.

The best results for etching *A. granosa* were obtained using a dilute solution of 0.05N HCl for 30 minutes at room temperature. Immediately after the surfaces had been etched, the acid was thoroughly rinsed off in running tap water. The surfaces of the sections were then left to air dry before the acetate peel could be made.

Acetate peel replicas were prepared by applying a piece of near molten replication material (Agar Scientific Ltd, UK), which had previously been immersed in ethyl acetate solvent for about 3 seconds, to the etched surface.

Once dry (ca. 10 mins.), acetate peels were removed, trimmed, and mounted on a slide beneath a cover slip and viewed in a light microscope.

4.2.3.2.2. Estimation of growth rates from microgrowth increments

The microgrowth patterns etched in the acetate peels of the sections of *A. granosa* and *A. antiquata* shells from the experimental animals were used to study the periodicity and rate of deposition of shell materials. Whereas microgrowth increments in the peels of sections selected from the monthly samples of clams were used to assess seasonal variations in shell growth and to investigate the effect of environmental factors on the microgrowth patterns.

For the experimentally marked blood clams, the number of microgrowth bands deposited between the notch and shell margin were counted. In order to examine whether there was a fortnightly spring-lunar variation in the growth rate, i.e. the width of individual microgrowth increments, the distance separating adjacent bands, was measured in 65 *A. granosa* grown for 64 days, using a calibrated eyepiece micrometer of 1μm accuracy. This time series data was then analysed by means of periodogram statistics (Warman, 1990) provided in the Perio Software (written by Alf Aagaard, Ecotoxicology, Institute of Biology, University of Odense, Denmark). Details of the programme are included in Appendix 4. The growth increment data set was tested for a period of between 8 and 20 days. A period of 14.8 days, an established standard semilunar periodicity, would indicate a fortnightly periodicity of shell formation (Naylor, 1982).

Having established the periodicity of the growth increments using the experimental marked-animals, the width of the last 14 bands to be deposited at the shell margin was measured in approximately 10 *A. granosa* from each monthly sample. The measurements were averaged and then plotted along

with the 95% confidence interval against time to investigate whether there was a seasonal variation in shell growth rate. The seasonal pattern of growth was then compared to the monthly measurements of environmental factors, viz. sediment temperature, salinity, chlorophyll-a and to the reproductive and condition indices described earlier in Chapters II and III respectively.

The shell surfaces of *A. granosa* and *A. antiquata* do not display any prominent growth rings which traditionally have been used to determine the age of temperate bivalve shells. Therefore, it was necessary to fully understand the periodicity of the microgrowth bands and the seasonal variations in shell microgrowth patterns, in order to identify any possible seasonal patterns of growth that could be used to determine the age of the blood clams. A cursory examination of the microgrowth patterns revealed a variation in the width of the microgrowth increments which was found to occur seasonally at the time of the major reproductive development (see later).

The shells were sectioned through their axes of normal height, and peel measurements were taken from the ventral margin at the surface of maximum growth which approximates to the perimeter p in Fig. 28D. The population modal progressions were however, obtained using data based on shell length measurements using calipers. Therefore, in order to enable comparisons to be made between shell length (SL, anterior-posterior axis) and the surface of maximum growth, a method for the measurement of the perimeter (p) was necessary. A piece of waxed cotton thread was placed on the external circumference of each shell from the umbo along the shell curvature towards the ventral margin perpendicular to the hinge plate. The thread was then measured with a vernier calliper to 0.01mm accuracy, as well as the shell length. Readings obtained in this way could be compared with measurements of linear shell length or height obtained with vernier

callipers (Richardson, 1987). The measurements were made for 32 individuals covering the widest size range of shells. A regression equation was then obtained relating the shell length and the perimeter, so that it was possible to convert the perimeter (p) to the length (SL). The relationship was:

$$\mathbf{p = 1.46 + 1SL}\ (n = 32,\ r = 0.987).$$

For the largest blood clams in the monthly samples, the position of the narrowing of the bands was marked on the slide using a very fine tipped drawing pen and the distance between the umbo and consequtive marks measured from the peel by means of a calibrated eyepiece micrometer of 1 μm accuracy. Size at age curves were plotted and K as well as L_∞ in the von Bertalanffy growth equation calculated using an asymptotic regression programme contained in the FISHPARM software package

4.3. Results

4.3.1. Allometric growth

A comparison of the allometric relationships between various size variables in the shells of *A. granosa* and *A. antiquata* are shown in Table 15. The first variables to consider are the allometric changes in the dimensions of the shell, i.e. shell length, width and shell height 2. The data shown in Table 15 indicates there are some similarities in the shell dimensions of the rounded form of *A. granosa* in Wedung (site 1) and Tapak (site 2). In Wedung, height 1 (axis of maximum radial growth) is isometric to both height 2 (normal height axis) and to shell length, whereas height 2 is negatively allometric to length. Similar isometric relationships were also observed at Tapak, although height 2 and length were isometrically related rather than negatively allometric with each other. This pattern of growth indicates that the shell is maintaining a rounded shape.

Table 15: Allometric relationships for log transformed shell measurements determined for *A. granosa* and *A. antiquata* from the three sites. Departures from isometry denoted as (+) for positive and (-) for negative allometry.

Site 1: Wedung, *A. granosa* rounded form, n= 145

Dependent	Independent	Allometry	a	b	r	SE_b	β	t	
Height 1	Length	isometric	0.813	0.999	0.990	0.0119	1	-0.0840	ns
Height 2	Length	-	0.887	0.957	0.979	0.0165	1	-2.6045	**
Height 1	Height 2	isometric	0.986	1.020	0.990	0.0119	1	1.6821	ns
Width	Length	+	0.588	1.050	0.965	0.0238	1	2.1017	**
Width	Height 1	+	0.728	1.050	0.976	0.0197	1	2.5355	**
Width	Height 2	+	0.693	1.080	0.975	0.0205	1	3.9082	**
Width	Hinge Plate	-	1.084	0.953	0.970	0.0201	1	-2.3418	**
Hinge Plate	Length	+	0.538	1.090	0.989	0.0135	1	6.6914	**
Hinge Plate	Height 1	+	0.698	1.080	0.989	0.0134	1	5.9880	**
Hinge Plate	Height 2	+	0.682	1.110	0.982	0.0176	1	6.2429	**
Weight	Length	-	0.0009	2.600	0.980	0.0444	3	9.0090	**
Weight	Height 1	-	0.0016	2.590	0.986	0.0365	3	11.239	**
Weight	Height 2	-	0.0014	2.680	0.987	0.0359	3	8.9236	**
Weight	Width	-	0.0043	2.400	0.983	0.0368	3	16.3043	**
Weight	Hinge Plate	-	0.0043	2.350	0.979	0.0409	3	15.8847	**

Site 1: Wedung, *A. granosa* elongated form, n= 200

Dependent	Independent	Allometry	a	b	r	SE_b	β	t	
Height 1	Length	+	0.655	1.050	0.993	0.0092	1	5.3468	**
Height 2	Length	isometric	0.670	1.000	0.987	0.0115	1	0.0000	ns
Height 1	Height 2	+	1.045	1.040	0.991	0.0101	1	3.9487	**
Width	Length	+	0.230	1.290	0.963	0.0253	1	11.4489	**
Width	Height 1	+	0.382	1.230	0.973	0.0205	1	11.1976	**
Width	Height 2	+	0.394	1.280	0.971	0.0130	1	12.4113	**
Width	Hinge Plate	+	0.395	1.260	0.955	0.0278	1	9.3559	**
Hinge Plate	Length	isometric	0.728	0.992	0.977	0.0155	1	-0.1561	ns
Hinge Plate	Height 1	-	1.136	0.930	0.969	0.0169	1	-4.1494	**
Hinge Plate	Height 2	isometric	1.149	0.973	0.970	0.0174	1	-1.5526	ns
Weight	Length	isometric	0.0001	3.010	0.976	0.0475	3	0.2105	ns
Weight	Height 1	-	0.0006	2.870	0.984	0.0369	3	-3.5345	**
Weight	Height 2	isometric	0.0006	2.990	0.983	0.0340	3	-0.2503	ns
Weight	Width	-	0.0062	2.280	0.989	0.0244	3	-29.4961	**
Weight	Hinge Plate	isometric	0.0006	2.920	0.961	0.0598	3	-1.3373	ns

Table 15 (continued)

Site 2: Tapak, rounded form *A. granosa*, n= 149

Dependent	Independent	Allometry	a	b	r	SE_b	β	t	
Height 1	Length	isometric	0.830	0.991	0.977	0.0180	1	-0.5005	ns
Height 2	Length	isometric	0.822	0.975	0.959	0.0236	1	-1.0602	ns
Height 1	Height 2	isometric	1.164	0.973	0.974	0.0186	1	-1.4524	ns
Width	Length	+	0.496	1.090	0.961	0.0258	1	3.4870	**
Width	Height 1	+	0.631	1.090	0.974	0.0209	1	4.3083	**
Width	Height 2	+	0.687	1.080	0.971	0.0219	1	3.6613	**
Width	Hinge Plate	isometric	0.963	0.980	0.970	0.0217	1	-0.9234	ns
Hinge Plate	Length	+	0.548	1.090	0.975	0.0203	1	4.4422	**
Hinge Plate	Height 1	+	0.715	1.080	0.982	0.0171	1	4.6811	**
Hinge Plate	Height 2	+	0.819	1.060	0.965	0.0238	1	2.5189	**
Weight	Length	-	0.0007	2.670	0.970	0.0557	3	5.9278	**
Weight	Height 1	-	0.0012	2.670	0.982	0.0423	3	7.8070	**
Weight	Height 2	-	0.0016	2.640	0.973	0.0515	3	6.9971	**
Weight	Width	-	0.0045	2.400	0.986	0.0334	3	13.9748	**
Weight	Hinge Plate	-	0.0035	2.400	0.972	0.0482	3	12.4585	**

Site 3: Bandengan, *A. antiquata*, n= 370

Dependent	Independent	Allometry	a	b	r	SE_b	β	t	
Height 1	Length	-	1.082	0.951	0.981	0.0097	1	5.0599	**
Height 2	Length	-	0.862	0.954	0.985	0.0087	1	5.3099	**
Height 1	Height 2	-	1.343	0.974	0.975	0.0116	1	2.0672	**
Width	Length	+	0.482	1.060	0.971	0.0138	1	-4.3572	**
Width	Height 1	+	0.483	1.090	0.965	0.0154	1	5.8556	**
Width	Height 2	+	0.594	1.110	0.973	0.0136	1	7.3421	**
Width	Hinge Plate	+	0.693	1.080	0.956	0.0172	1	4.6431	**
Hinge Plate	Length	-	0.802	0.955	0.980	0.0100	1	4.4865	**
Hinge Plate	Height 1	-	0.836	0.968	0.963	0.0141	1	-2.2679	**
Hinge Plate	Height 2	-	0.983	0.972	0.978	0.0110	1	2.5783	**
Weight	Length	-	0.0004	2.790	0.982	0.0282	3	7.4600	**
Weight	Height 1	-	0.0004	2.86	0.975	0.0342	3	-4.0996	**
Weight	Height 2	-	0.0007	2.90	0.987	0.0239	3	-4.1789	**
Weight	Width	-	0.0034	2.55	0.984	0.0240	3	-18.7344	**
Weight	Hinge Plate	-	0.0010	2.84	0.973	0.0348	3	-4.5924	**

y and x are the dependent and independent variables in the equation log y = log a +b logx. Intercept is denoted as a, β and b (slope of the regression lines) are the coefficients of isometry and allometry respectively; r is the coefficient of correlation, SE_b is the standard error of b, the slope, and t is the calculated value resulting from the t-test. Significance levels: ns is not significant, * $P<0.05$, ** $P< 0.01$.

The elongated form of *A. granosa* from site 1, i.e. Wedung showed different relationships between the shell dimensions (Table 15). In this form, height 1 and length as well as height 1 and height 2 were positively allometric whereas height 2 and length were isometric. This positively allometric relationship with height indicates that this dimension is increasing relatively faster than length and thus maintaining an elongate form to the shell. *A. antiquata* on the other hand showed a negatively allometric relationship between length and height indicating that the anterior-posterior axis is increasing faster than the height.

In both *A. granosa* and *A. antiquata*, maximum shell width is situated more to the ventral shell margin, thereby lowering the centre of gravity and providing a broad base for burrowing or byssal attachment. For both forms of *A. granosa* from Wedung as well as for the population for Tapak, width is positively allometric to length and the two height axes (Table 15). Thus suggesting that the clams tend to grow faster laterally to maintain their globose shape.

The hinge plate in the rounded form of *A. granosa* perhaps act as an anchor to balance the position in the burrow thus improving the stability of the bivalve. In Wedung the width of the rounded shell is negatively allometric with respect to this plane of articulation, whilst in Tapak it is isometric. In the elongated form and in *A. antiquata* width and hinge plate dimensions are positively allometric. Development of the hinge plate could perhaps be related to the nature of the substratum.

Wedung has finer sediment particles than Tapak and blood clams may need to develop the auricle-like hinge plate structure to stabilise their position within the burrow made by the slender apex of the anterior region of the shell. In Tapak, the bottom sediment is coarser and has a greater proportion of fine sand with less silt and clay fractions than the sediment in

Wedung (Table 5, Chapter II). In addition, the sediment at Tapak has an admixture of small twigs and bamboo pole fragments. Whilst this kind of sediment may be difficult to burrow into, it will hold the bivalves within the sediment and prevent them from sinking too deep. However, the discrepancy between the rounded form at Wedung and Tapak was not all that great, since the hinge plate is found to have a positive allometric relationship to shell length and both heights. These relationships, where the hinge plate increases relatively faster in size than the length and height, may serve as further evidence that the hinge plate may be important for the rounded form of *A. granosa* at Wedung and Tapak. For the elongated form the relationships between these dimensions varied from negatively allometric to isometric, whereas those of *A. antiquata* at Bandengan have negatively allometric relationships (Table 15).

The shell weight of the rounded clams in Wedung and Tapak exhibit negatively allometry to the length, height, width and the hinge plate dimensions. This indicates that the animals tend to grow faster in all of those linear axes achieving a greater surface area/volume ratio by maintaining a light weight shell.

The umbones in the elongated form of *A. granosa* are located almost centrally in the axis of the hinge plate, whilst in the rounded clams they are positioned slightly more towards the anterior part of the shell. Also, the elongated form has a finer row of tubercles along its radial ribs compared to that in the rounded form. The maximum size collected for the elongated form never exceeded 40.6mm in length whilst the maximum size of the rounded one was 60mm. However, since the samples did not contain a similar size range, a statistical comparison between them using GLM could only be carried out using those rounded form specimens which were comparable in size to the elongated ones (see Table 16).

Table 16: Coefficients of allometry and covariance analysis for various combinations of size parameters in the rounded and elongated forms of *A. granosa* from Wedung. Divergences from isometry denoted as (+) for positive and (-) for negative allometry.

Variable		Coefficient of allometry of:				Comparison of (F):	
Dependent	Independent	Rounded		Elongated		Intercept	Slope
		b	Allometry	b	Allometry		
Height 1	Length	0.999	isometric	1.050	+	17.94 **	12.18 **
Height 2	Length	0.957	-	1.000	isometric	15.63 **	4.00 **
Height 1	Height 2	1.020	isometric	1.040	+	0.66 ns	2.09 ns
Width	Length	1.050	+	1.290	+	44.52 **	31.59 **
Width	Height 1	1.050	+	1.230	+	30.86 **	21.73 **
Width	Height 2	1.080	+	1.280	+	22.62 **	26.91 **
Width	Hinge Plate	0.953	-	1.260	+	76.40 **	62.63 **
Hinge Plate	Length	1.090	+	0.992	isometric	20.05 **	24.87 **
Hinge Plate	Height 1	1.080	+	0.930	-	54.11 **	52.85 **
Hinge Plate	Height 2	1.110	+	0.973	isometric	45.32 **	27.55 **
Weight	Length	2.600	-	3.010	isometric	48.70 **	34.35 **
Weight	Height 1	2.590	-	2.870	-	31.53 **	22.27 **
Weight	Height 2	2.680	-	2.990	isometric	22.12 **	29.99 **
Weight	Width	2.400	-	2.280	-	1.55 ns	0.93 ns
Weight	Hinge Plate	2.350	-	2.920	isometric	73.04 **	60.93 **

The dependent and independent variables are y and x respectively in the equation log y = log a +b logx. Slopes of the regression lines as coefficient of allometry denoted as b. Significance levels: ns is not significant, * $P<0.05$, ** $P< 0.01$.

Table 17: Coefficients of allometry and covariance analysis for various combinations of size parameters of the rounded form of *A. granosa* (Wedung) and *A. antiquata* (Bandengan). Divergences from isometry denoted as (+) for positive and (-) for negative allometry.

Variable		Coefficient of allometry of:				Comparison of (F):			
Dependent	Independent	*A. granosa*		*A. antiquata*		Intercept		Slope	
		b	Allometry	b	Allometry				
Height 1	Length	0.999	isometric	0.951	-	27.94	**	8.44	**
Height 2	Length	0.957	-	0.954	-	0.01	ns	0.47	ns
Height 1	Height 2	1.020	isometric	0.974	-	16.85	**	1.54	ns
Width	Length	1.050	+	1.060	+	2.21	ns	0.02	ns
Width	Height 1	1.050	+	1.090	+	21.59	**	1.09	ns
Width	Height 2	1.080	+	1.110	+	5.12	**	0.71	ns
Width	Hinge Plate	0.953	-	1.080	+	28.14	**	22.31	**
Hinge Plate	Length	1.090	+	0.955	-	49.71	**	73.35	**
Hinge Plate	Height 1	1.080	+	0.968	-	7.03	**	35.44	**
Hinge Plate	Height 2	1.110	+	0.972	-	20.61	**	29.36	**
Weight	Length	2.600	-	2.790	-	16.87	**	11.47	**
Weight	Height 1	2.590	-	2.86	-	60.09	**	23.19	**
Weight	Height 2	2.680	-	2.90	-	39.11	**	36.70	**
Weight	Width	2.400	-	2.55	-	5.61	**	17.77	**
Weight	Hinge Plate	2.350	-	2.84	-	80.51	**	93.33	**

The dependent and independent variables are y and x respectively in the equation log y = log a +b logx. Slopes of the regression lines as coefficient of allometry denoted as b. Significance levels: ns is not significant, * $P<0.05$, ** $P< 0.01$.

Table 18: Coefficients of allometry and covariance analysis for various combinations of size parameters of the elongated form of *A. granosa* (Wedung) and *A. antiquata* (Bandengan). Divergences from isometry denoted as (+) for positive and (-) for negative allometry.

Variable		Coefficient of allometry of:				Comparison of (F):	
Dependent	Independent	*A. granosa*		*A. antiquata*		Intercept	Slope
		b	Allometry	b	Allometry		
Height 1	Length	1.050	+	0.951	-	215.57 **	197.02 **
Height 2	Length	1.000	isometric	0.954	-	177.39 **	165.20 **
Height 1	Height 2	1.040	+	0.974	-	9.14 **	4.76 **
Width	Length	1.290	+	1.060	+	234.57 **	230.23 **
Width	Height 1	1.230	+	1.090	+	4.97 **	17.08 **
Width	Height 2	1.280	+	1.110	+	15.14 **	27.89 **
Width	Hinge Plate	1.260	+	1.080	+	22.15 **	21.06 **
Hinge Plate	Length	0.992	isometric	0.955	-	169.04 **	166.32 **
Hinge Plate	Height 1	0.930	-	0.968	-	8.32 **	1.19 ns
Hinge Plate	Height 2	0.973	isometric	0.972	-	6.32 **	0.79 ns
Weight	Length	3.010	isometric	2.790	-	227.77 **	218.65 **
Weight	Height 1	2.870	-	2.86	-	8.99 **	3.62 ns
Weight	Height 2	2.990	isometric	2.90	-	5.60 **	2.97 ns
Weight	Width	2.280	-	2.55	-	33.25 **	44.57 **
Weight	Hinge Plate	2.920	isometric	2.84	-	0.70 ns	2.17 ns

The dependent and independent variables are y and x respectively in the equation log y = log a +b logx. Slopes of the regression lines as coefficient of allometry denoted as b. Significance levels: ns is not significant, * $P<0.05$, ** $P< 0.01$.

It is clear from Table 15 that *A. antiquata* does not display any isometric relationships between the various shell dimensions. This species tends to grow its transversal axis (length) faster than the radial one (normal height). In addition, it expands laterally faster than it does transversally. These distinct characteristics combined with a negative allometric growth between shell weight and the rest of the linear dimensions (length, width, hinge plate, and height) ensure that the clams produce a light-weight globular shell. Moreover, in contrast to *A. granosa*, the growth of the hinge plate is slower than the length, height, and width of the shell, perhaps implying that this plate is not functionally as important for stabilising the bivalve in the sediment as it is for *A. granosa.*

An analysis of covariance running 'form' as a single covariate upon the rounded and elongated forms of *A. granosa* from Wedung (Table 16), the rounded form of *A. granosa* and *A. antiquata* (Table 17) and, the elongated form of *A. granosa* and *A. antiquata* (Table 18), revealed more differences between the shell dimensions of the rounded and elongated form of *A. granosa.* Almost all, 13 out of 15 comparisons, of the shell dimensions showed a highly significant difference ($P<0.01$) between either the intercepts or slopes of the regression lines. Whereas comparing each of the dimensions between *A. granosa* and *A. antiquata* resulted in significant differences in 10 out of the 15 measurements (Tables 16 to 18).

As shown in Table 16, almost all the allometric relationships between comparable size-ranges of the two forms of *Anadara*, i.e. the rounded and the elongated form, are significantly different from each other. Nevertheless, for the rounded form normal height is isometric to the axis of maximum height (height 1) yet is positively allometric for the elongated form. However, both are not significantly different when the regression slopes of the relationships are compared. This suggests that a positive divergence from

isometry in the elongated form is not in fact very obvious. Similar findings were also found for shell weight and width, in which both showed a negative allometric relationship.

Shown in Table 17 is a comparison of the coefficients of allometry of the rounded form of *A. granosa* from Wedung and *A. antiquata* from Bandengan. Both species show that their length increases at a faster rate than their normal (vertical) height. At the same time they are expanding their width laterally faster than any increase in the other linear axes. Although in both species shell weight is negatively allometric to shell length, *A. antiquata* presumably produces a heavier shell than the rounded *A. granosa* as indicated by such coefficients of allometry which are closer to isometry and significantly different from those of *A. granosa.*

When comparing the elongated form of *A. granosa* to *A. antiquata* (Table 18), it is apparent that even though both are growing faster laterally, the elongated form of *A. granosa* becomes more globular than the other as their size increases. This is indicated by the significant difference between shell width and the linear dimensions, shell length, height and length of the hinge plates for which the values obtained for the elongated *A. granosa* are closer to isometry. Moreover, *A. antiquata* of the same length will have less heavier shells and a less pronounced hinge plate compared to the elongated *A. granosa.* Therefore, in general the elongated form of *A. granosa* has the heaviest shell and the most globular shell outline amongst them. Whereas the rounded form of *A. granosa* has the lightest shell which remains the same width as *A. antiquata* whilst they are increasing in size.

4.3.2. Absolute Growth

4.3.2.1. The length frequency distribution

The length frequency histograms for the monthly samples are summarised in Figure 29 with the details shown in Figures 30-32. The population in Wedung showed two periods of major recruitment each in August 1991 and August 1992 (Fig. 30), besides another two recruitments in November and March. The following year, 1993, recruitment occurred a month earlier, with individuals entering the population at a size of 8-12 mm. After recruitment in August 1991 the number of spat diminish in the population during the 12 months which elapse until August 1992 upon which a new recruitment appears. Similarly, a new cohort dominated the population in July-August 1993. When they first appear in the population in August and September, the recruits consist of a single prominent modal size, but as they grow, other recruits join the population as in October 1991 and March 1992 at a size of 19-20mm (Figs. 29A, 30). Another heavy recruitment of clams with a single modal size of 10-14mm appeared from August-September in 1992 and from July-August 1993 in Wedung.

Following the separation of the polymodal distribution into its component modes using the method of Bhattacharya, recruits arriving into the population between October 1991 and March 1992 can be traced until July 1992 and January 1993 respectively. It is clear however, that in general most of the blood clams in the population at Wedung did not live longer than 10 months from the time when they first appeared as recruits (Fig. 30). Both cohorts A and D emerge as major recruitments in August 1991 and 1992 whilst cohort G appears later in July and August 1993. Of those blood clams in cohort B which appeared in October-November 1991 only a very few representatives remained by July 1992, some 10 months after appearing in the population.

Figure 29: Population growth curves resolved by the aid of Bhattacharya's method. Bars represent the Standard Deviation of the average.

A. Population growth curves of *A. granosa* at the Wedung population, showing 5 different cohorts. Major recruitments are confined to the mid dry season in between July-September.

B. Population growth curves of *A. granosa* at Tapak assessed from combined four-monthly data. Three different cohorts are resolved with negligible recruitments.

C. Population growth curves of *A. antiquata*, Bandengan, showing 4 different cohorts with 2 recruitments in February 1992 and later in August 1992.

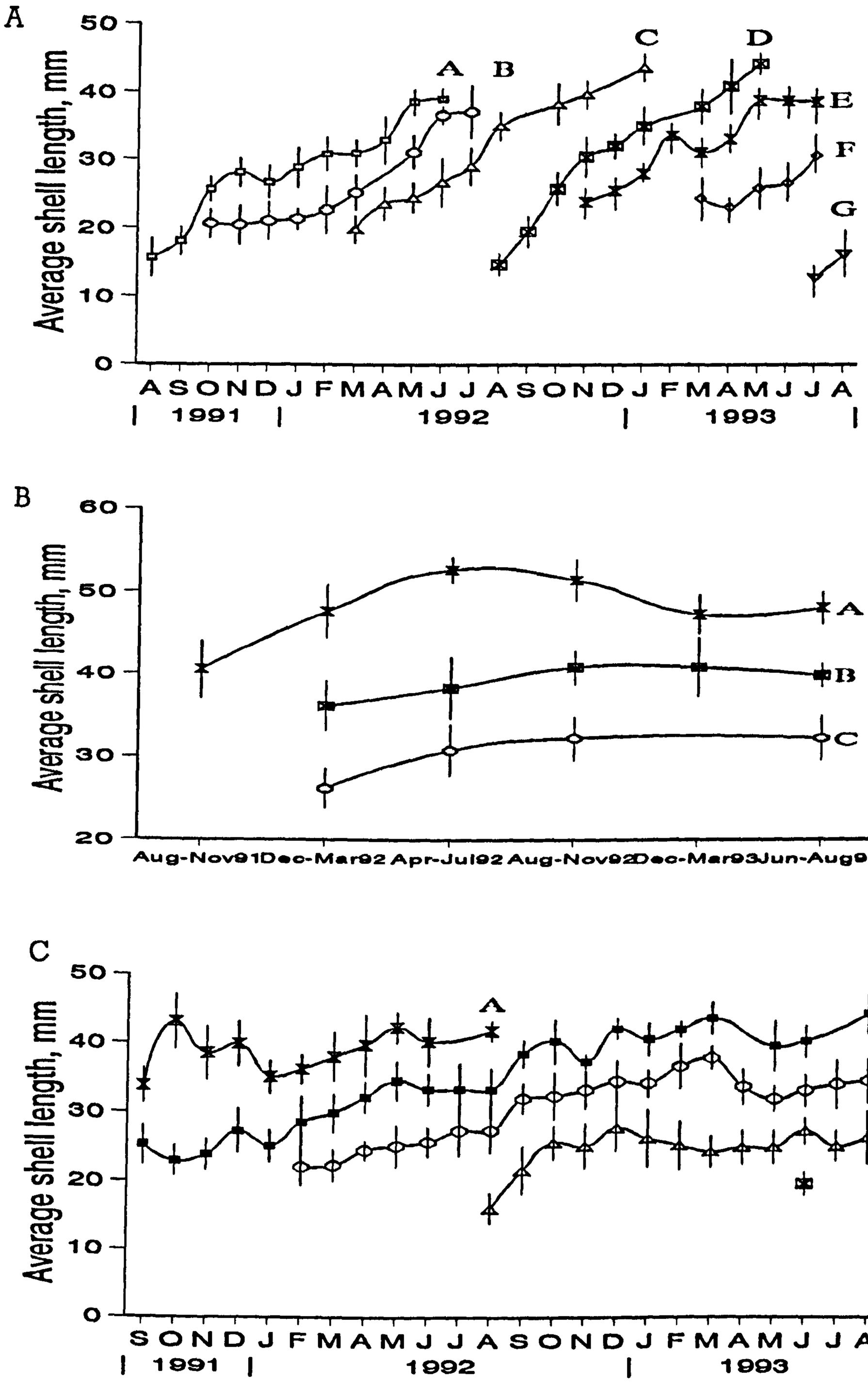

A
Average shell length, mm
50
40
30
20
10
0
A
B
C
D
E
F
G
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
Average shell length, mm
60
50
40
30
20
A
B
C
Aug-Nov91
Dec-Mar92
Apr-Jul92
Aug-Nov92
Dec-Mar93
Jun-Aug93
C
Average shell length, mm
50
40
30
20
10
0
A
B
C
D
S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

Figure 30: Length frequency distributions of monthly samples of *A. granosa* from Wedung. Numbers represent total number of individuals. Letters denote estimated cohorts fitted using the method of Bhattacharya

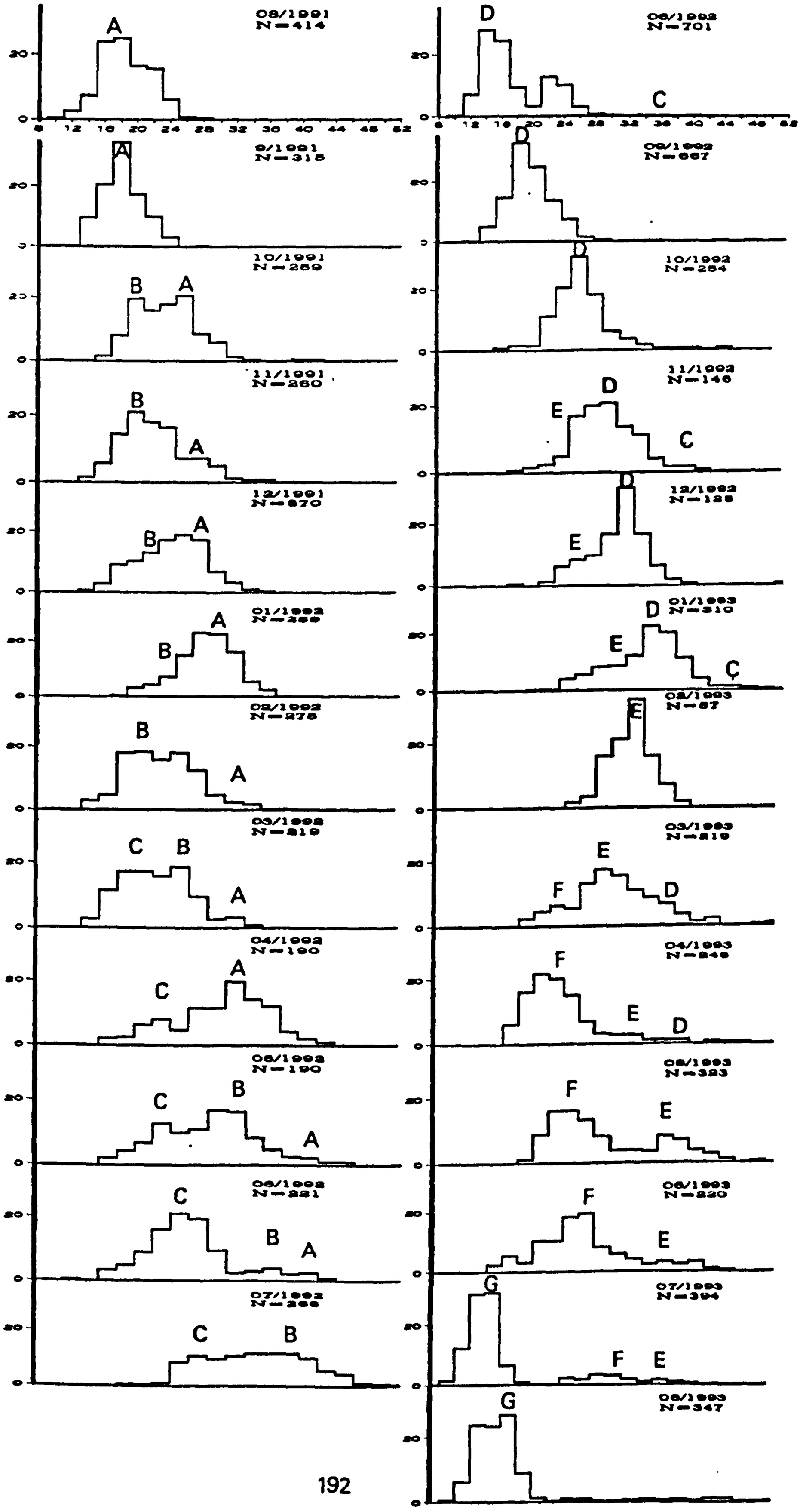

08/1991
N=414
09/1991
N=315
10/1991
N=269
11/1991
N=260
12/1991
N=270
01/1992
N=269
02/1992
N=276
03/1992
N=219
04/1992
N=190
05/1992
N=190
06/1992
N=221
07/1992
N=266
06/1992
N=701
09/1992
N=667
10/1992
N=254
11/1992
N=146
12/1992
N=125
01/1993
N=310
02/1993
N=87
03/1993
N=219
04/1993
N=249
05/1993
N=323
06/1993
N=320
07/1993
N=394
08/1993
N=347

Figure 31: Length frequency distributions for four-monthly population samples of *A. granosa* from Tapak. Numbers represent total number of individuals. Letters denote estimated cohorts fitted using the method of Bhattacharya. Samples were not available for April and May 1993.

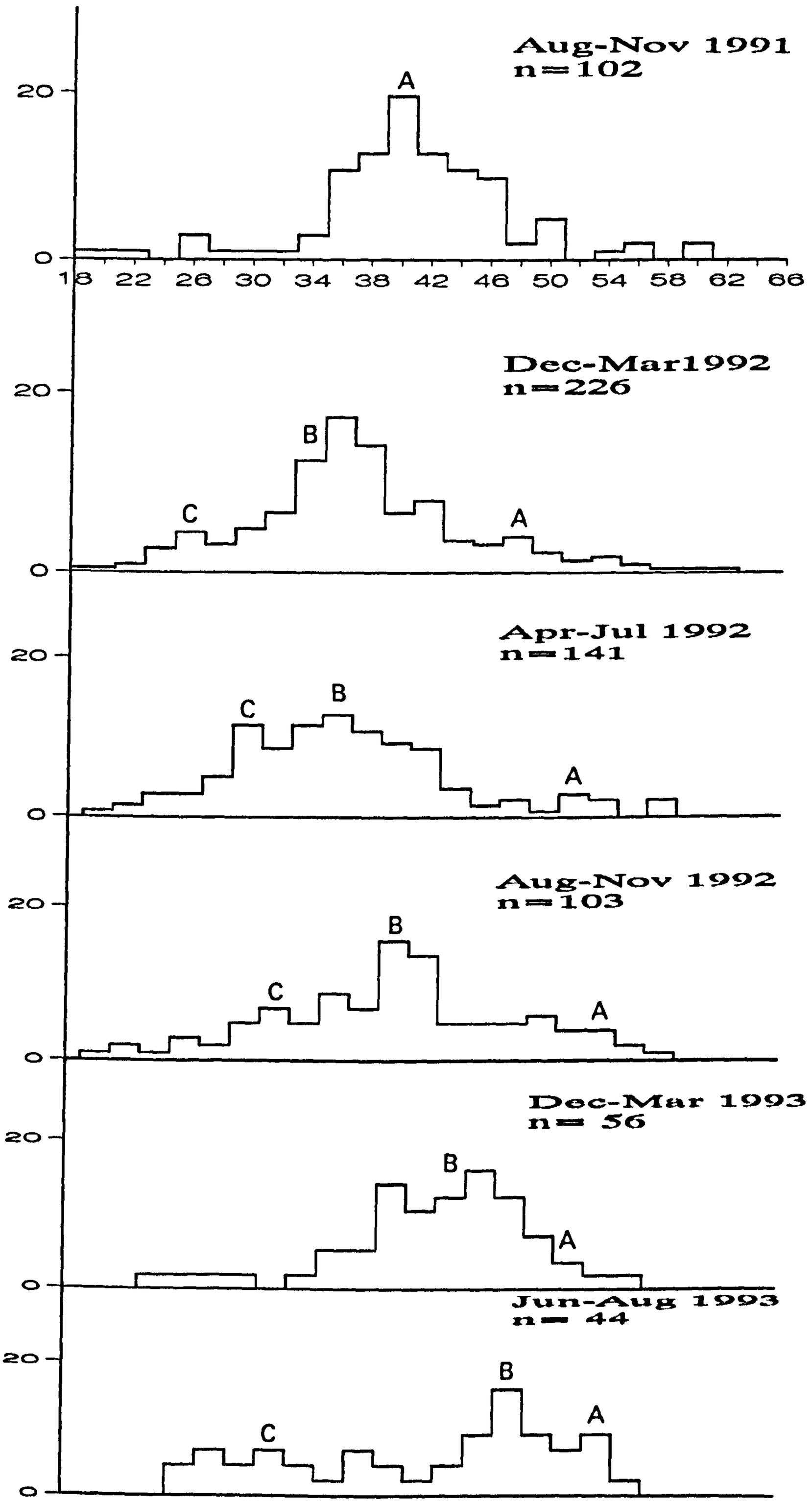
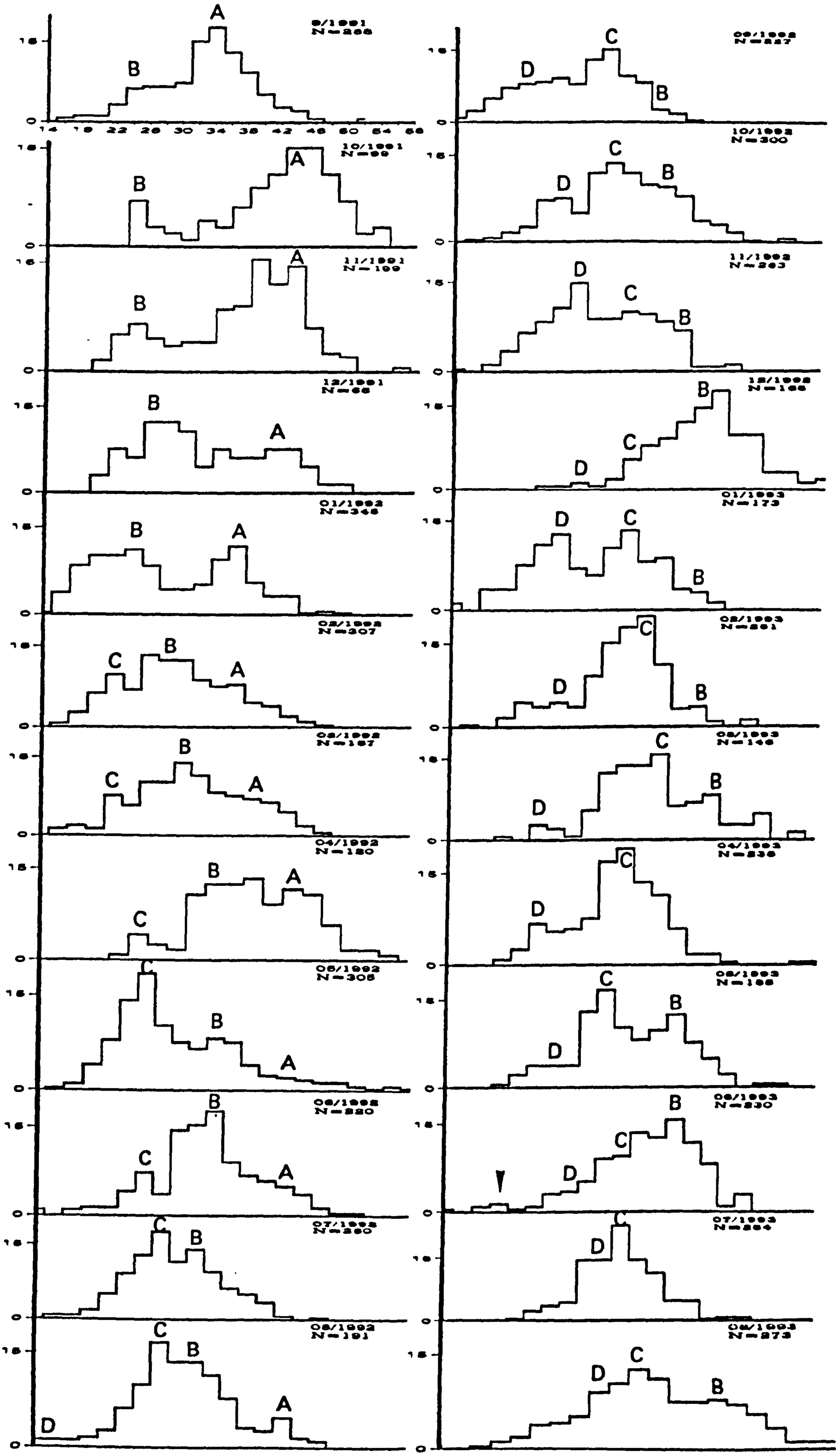

Aug-Nov 1991
n=102
A
20
0
18 22 26 30 34 38 42 46 50 54 58 62 66
Dec-Mar1992
n=226
B
C
A
Apr-Jul 1992
n=141
C
B
A
Aug-Nov 1992
n=103
B
C
A
Dec-Mar 1993
n= 56
B
A
Jun-Aug 1993
n= 44
B
C
A

Figure 32: Length frequency distributions of monthly samples of *A. antiquata* from Bandengan. Numbers represent total number of individuals. Letters denote estimated cohorts fitted using the method of Bhattacharya


A
B
C
D
0
16
14 18 22 26 30 34 38 42 46 50 54 58
09/1991
N=208
10/1991
N=99
11/1991
N=199
12/1991
N=66
01/1992
N=348
02/1992
N=307
03/1992
N=187
04/1992
N=180
05/1992
N=305
06/1992
N=320
07/1992
N=280
08/1992
N=191
09/1992
N=227
10/1992
N=300
11/1992
N=203
12/1992
N=166
01/1993
N=173
02/1993
N=201
03/1993
N=146
04/1993
N=230
05/1993
N=188
06/1993
N=230
07/1993
N=264
08/1993
N=273

The majority of cohort C had similarly disappeared after 5 months (March-July 1992, Fig. 29A), although the method used can distinguish a few specimens of this cohort until January 1993 (Fig. 30).

An abrupt decrease in size disturbed the modal size of cohort E in March-April 1993 (see Fig. 29A) which was caused by the blood clams collected in March 1993 containing relatively a number of small individuals (Fig. 30). Where these blood clams came from is not known, but their presence could be attributed either to sampling error or to the severe environmental conditions prevalent at that time which may have washed the animals from the adjacent populations into the survey area. In December 1992 the previous year for example, there was prolonged flooding followed by increased fishing activitiy for blood clams. Thirty five local fishermen were observed to be actively collecting blood clams during the sampling period in January 1993. The increase in fishing activity coincided with another big flood inundating the estuaries a month later in February 1993. Although this second flood was less damaging to the area than the earlier one, the effect of the flooding would certainly have been stressful for sedentary species. After the flood the bottom sediment became firm and compact underfoot, to such an extent that one could easily walk on it. These compacted sediments may have affected the blood clams and given rise to the anomalous size distribution in March 1993.

After the major recruitment in August 1992 in Wedung, no further spatfall occurred until November 1992. The modal size of the population shifted from 34mm in February 1993 to 30mm in March 1993, then a peak of 22-24mm individuals appeared in March 1993 dominated the population in April 1993 (cohort F). Even so, this mode was only obvious for five months until July 1993 before a new recruitment arrived and left cohort F as a remnant in July 1993. Between June and August 1993, heavy fishing activity

for *A. granosa* was observed in Wedung. In the subtidal areas further seaward from the survey area, between 4-16 boats were observed to be collecting the spat for transfer to tambaks. In addition, 24 people were also manually collecting blood clams in the sampling areas.

With the knowledge that the natural population in Wedung is regularly disturbed, growth estimations particularly in months when the beds are heavily exploited would only give a biased value. Nevertheless, assuming that within the first three months following heavy recruitments the population modal size was relatively undisturbed, the mode of the length frequency distributions moved from 16mm in August 1991 to 26mm in October 1991. This was similarly repeated the following year in the August to October 1992 samples when the mode moved from 14mm to 26mm (Fig. 30). It can be seen from the length frequency histograms that in 1991 the August mode did not differ from the September one perhaps because they were only a fortnight apart.

Investigation of the modal progression during the first six months after recruitment, i.e. from August to January, the mode at 16mm in August 1991 moved to 30mm in January 1992. Subsequently, in August the following year the mode moved from 14mm to 34mm within the same six months time. These were giving an average size increment of 2.3mm.month^{-1} in August 1991-January 1992 and 3.3mm.month^{-1} during August 1992-January 1993 (Figs. 29A & 30). Thereafter, it is difficult to estimate their growth rate merely from the modal progression analysis, since fishing activities and floods in February 1993 may have influenced the mode of the length frequency distribution.

Recruitment of *A. granosa* appears to have been negligible in the Tapak population. The method of Bhattacharya distinguished 3 cohorts (i.e. cohorts A-C in Figs. 29B and 31). Cohort C which appeared in December-March

1992 may be compared to cohorts E and F at Wedung population which also emerge within the similar period of time, i.e. November 1991 and March 1992 respectively (Figs. 29A and 30).

The length frequency histograms for *A. antiquata* from Bandengan (Figs. 29C & 32) show that in particular months, e.g. December 1992 and June-July 1993, the modes have merged to produce a unimodal distribution. However, for the rest of the months Bhattacharya's method was able to resolve 4 major cohorts, A to D.

Summarising the results obtained from the Bhattacharya method, an examination of the rate of increase of the modal size classes with time shown in Figure 29C suggests that the growth rate of *A. antiquata* in Bandengan (Fig. 32) is slower than that of *A. granosa* in Wedung (Figs. 29A and 30), although it would appear that blood clams from Bandengan are longer lived than those at Wedung. The method of Bhattacharya further identified two recruitments at Bandengan during February and August 1992; a smaller recruitment appeared in June 1993 (arrowed) which then merged during the following months with the adult populations (see Figs. 29C and 32). The mode of 24mm in population B in September 1991 moved to 30mm in February 1992, and from 20mm to 26mm in the same time at the following year for population D; both distributions indicate a growth rate of 1mm.month^{-1} for *A. antiquata*.

4.3.2.2. Interpretation of the microgrowth patterns

Viewed in the light microscope, acetate peels replicas of polished and etched shell sections revealed that the shells of *A. granosa* and *A. antiquata* are composed of 3 shell layers, i.e. 1) an outer periostracum, 2) an outer complex crossed lamellar layer and 3) an inner complex crossed lamellar layer (Fig. 28, see also Taylor et al, 1969). Plates 7 and 8 illustrate the appearance of radial sections of the periostracum and the outer crossed

lamellar layer and the microgrowth patterns. Fine, dark growth bands (GBO) bisect the outer crossed lamellar layer and separate wider, more translucent growth increments (Fig. 28 and Plates 7 & 8).

File marking of *Anadara* resulted in the formation of a deep cleft in the outer surface of the shell (see Plate 7A-E) to which all subsequent shell growth and growth band deposition could be related. Both *A. granosa* and *A. antiquata* showed evidence of shell growth after file marking. Most of the clams survived the shock of filing, although at one site, Tapak, there was more than 80% mortality of the marked and transplanted group. The growth banding in *A. antiquata* was weaker in definition (Plate 7D) than that observed in *A. granosa*, and for that reason the growth pattern in *A. antiquata* could not be used to study the growth of the shell. *A. granosa* from Wedung, however, showed a high rate of survival after transplantation into the shallow subtidal for 14, 36 and 64 days.

The mixed semidiurnal tidal pattern in the area of study has a predominant daily component, so that the number of tides is approximately equal to the number of days (see Fig. 34A and Table 19), therefore the tidal periodicity of deposition of the microgrowth bands can be referred to, in a general way, as daily bands. Counts of the number of growth bands deposited in the shells of *A. granosa* during the three experimental periods are summarised in Table 19.

Further analysis of the periodicity of the microgrowth bands using χ^2 goodness of fit test between the number of days in an experiment (which is approximately equal to the number of expected low tides) and the average number of bands observed, revealed that there was no significant difference ($P<0.001$) at 14 days ($\chi^2= 0.583$; df= 11), 36 days ($\chi^2= 8.94$; df= 16) and 64 days ($\chi^2= 55.606$; df= 60).

Table 19: Numbers of growth bands deposited in the outer crossed lamellar layer of *Anadara granosa* at Wedung for several intervals of time

Duration of growth experiment in 1993	No. of days of experiment	No. of low tides	No. of clams examined	No. of bands deposited between cleft and shell margin, ± Std. Error
5 June - 19 June	14	14	12	12.75 ± 0.37
5 June - 11 July	36	36	17	30.29 ± 1.28
5 June - 8 August	64	64	61	54.35 ± 2.12

Plate 7A shows an acetate peel of a blood clam file marked and grown for 14 days at Wedung, 13 bands can clearly be seen in the photomicrograph, whilst band 14 can only be observed directly in the microscope. Plate 7(B, C and E) show acetate peels of three blood clams file marked and grown for 64 days at Wedung. All these clams show a wide pattern of banding immediately after marking, but the growth of the clam in Plate 7B has all but stopped by the 29th day (6.14% of the individuals in the sample) whilst growth of the clam in Plate 7C slows down at day 44 (78.5% of individuals in the sample) and thereafter shell deposition continues at a slower rate. Only the clam shown in Plate 7E has deposited 64 widely spaced bands (15.36% of individuals in the sample).

Measurement of the width of each daily growth increment between the file mark and the shell margin provides an insight into the individual variation in band deposition. There was a good correspondence between the number of bands laid down in the blood clams grown for 14 days and the number of days of the experiment (Table 19 and Fig. 33A). The confidence interval about the width of each growth increment does not appear to vary appreciably between day 1 and 14 (Fig. 33A). However, clams grown for 36 days show some variation between the expected and observed number of bands (Table 19) as shown by the large standard error. A plot of the width of the daily increment against time for 17 individuals shows that there is an increase in the 95% confidence interval at around day (band) 24 in blood clams grown for 36 days (Fig. 33B).

The measurement of the daily increment width in a relatively large number of blood clams (78.5% or n= 51) shows there is generally a marked reduction in shell growth about day (band) 20. However, a number of individual blood clams continued to grow normally during the 64 days of the experiment (e.g. Plate 7E). The daily variation in increment width of 10 blood clams (15.36%)

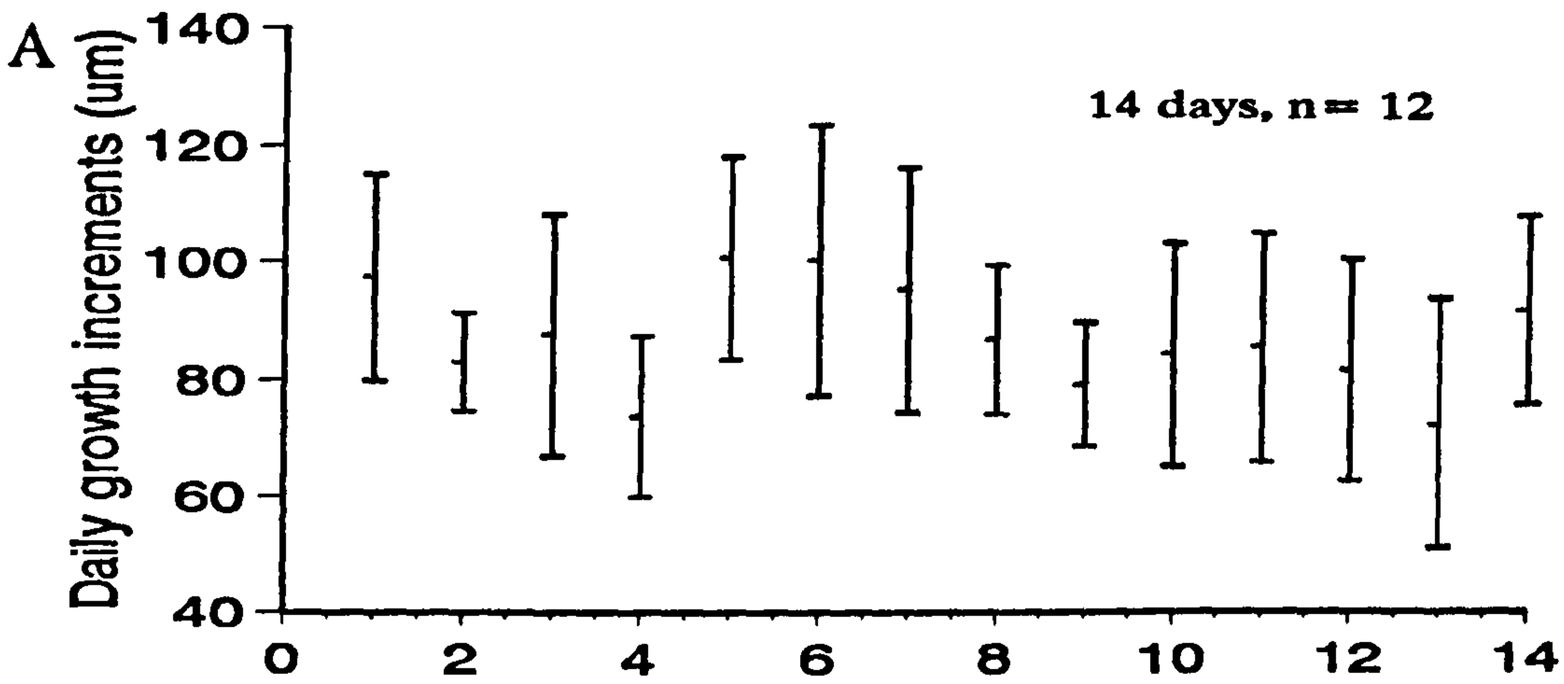
Figure 33: Variation in the width of the daily growth increments of file-marked *A. granosa* of 17.7-24.5mm shell length which were file marked and transplanted for several different durations at Wedung. Bars represent the 95% confidence intervals.

A and B. The pattern of daily increment width deposited during 14 and 36 days of the experiment.

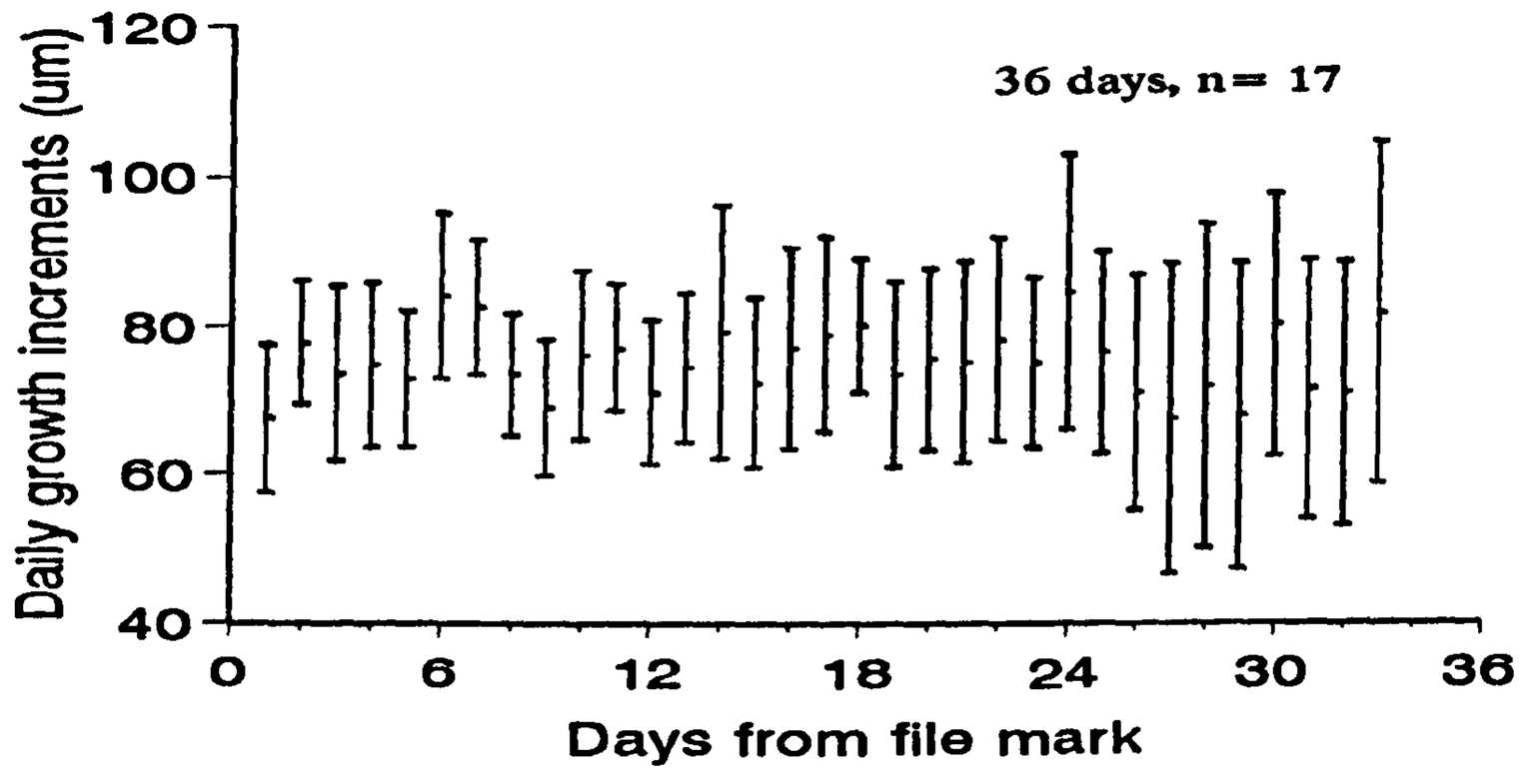
C. The pattern of daily increment width deposited during 64 days of the experiment. The steady decline in width of the increments was due to the onset of spawning during June to August 1993.

D. Analysis of periodogram statistics for the time series data shown in C, to show there is no significant spring-neap lunar pattern of growth.

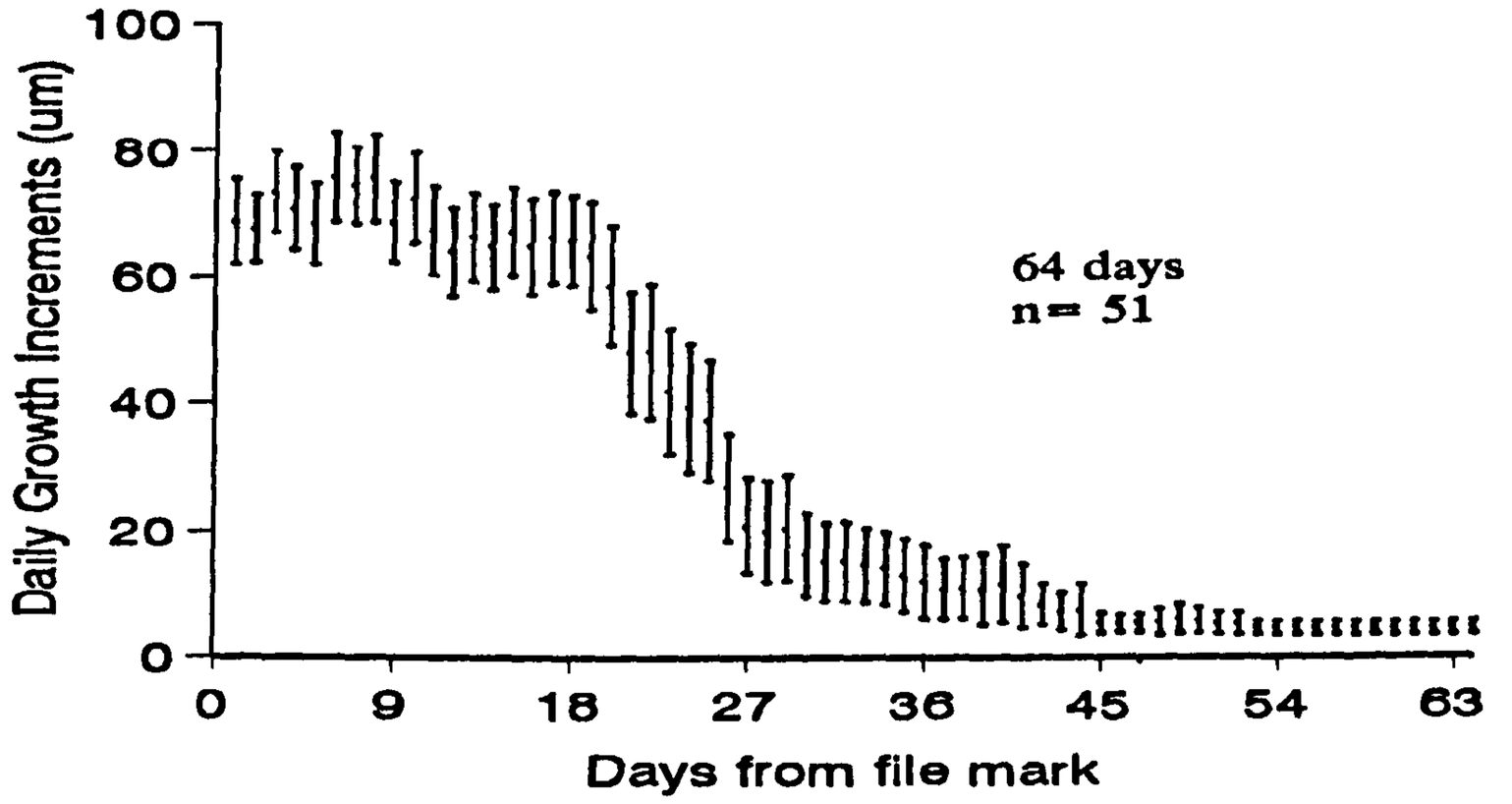
A


Daily growth increments (um)
140
120
100
80
60
40
14 days, n= 12
0
2
4
6
8
10
12
14
Days from file mark

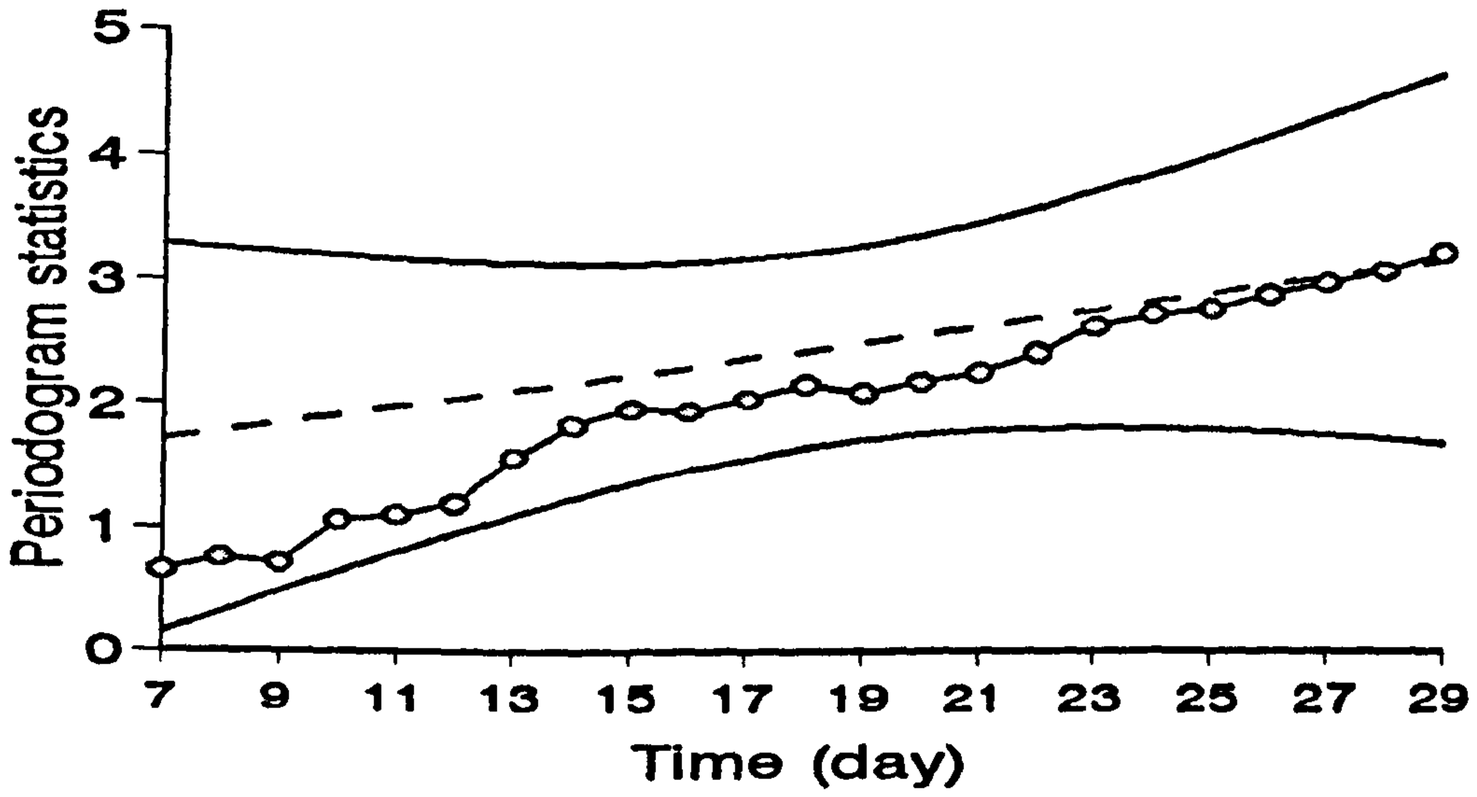
B


Daily growth increments (um)
120
100
80
60
40
36 days, n= 17
0
6
12
18
24
30
36
Days from file mark

C


Daily Growth Increments (um)
100
80
60
40
20
0
64 days
n= 51
0
9
18
27
36
45
54
63
Days from file mark

D


Periodogram statistics
5
4
3
2
1
0
7
9
11
13
15
17
19
21
23
25
27
29
Time (day)

which grew normally during the 64 day period is shown in Figure 34B. An apparent periodic variation in increment width is present in the pattern, with minimum growth occuring during or a few days after maximum spring tides (compare Figs. 34 A and B). When the pattern of increment widths was further investigated using periodogram analysis, the resultant plot was produced (Fig. 34C). A significant decrease in increment width occurred every 14-15 days as shown by the periodogram statistics in Figure 34C. Thus the spring-neap lunar cycle has in some way influenced shell deposition in the blood clams growing rapidly during the 64 days experiment. However, when the pattern of daily increment widths of the blood clams whose growth had slowed down appreciably during the 64 days were analysed using periodogram analysis, no apparent spring-neap lunar pattern could be discerned (Fig. 33D). A possible cause was sought as to why the growth rate of some of the clams had slowed down during the 64 days yet others had continued to deposit shell normally.

It has been shown in Chapter III that in 1993 the major spawning period for *A. granosa* at Wedung was between June and August (see Figs. 14 and 26A and C). It is therefore highly likely that the gonads of the blood clams grown in the growth experiment at Wedung for 64 days during June and August 1993 were in the process of reaching sexual maturity and were ready to spawn, and that individuals were reaching this state at different rates. The onset of reproduction presumably affects shell deposition. Subsequently, the variation in increment widths of those clams shown in Plates 7B, C and E probably reflects the different rates at which the gonads of these individuals reached sexual maturity. The photomicrographs in Plate 8A and B are taken from blood clams of 20.4mm and 25.1mm collected in August 1992 and July 1992. The acetate peels show narrow and wide increments respectively at their shell margins.

Plate 7: Photomicrographs of acetate peels from shell sections of *A. granosa* grown in the field for 14 and 64 days in Wedung and 118 days for *A. antiquata* in Bandengan. Growth from left to right. Scale bars = 500μ m.

A. *A. granosa* grown for 14 days, showing 13 clear growth bands. Band 14 can only be seen directly in the microscope. Small arrow shows position of file mark.

B. *A. granosa* grown for 64 days. The clam stopped growing at day 29th (numbered arrow) of the experiment and formed a block of unresolved bands.

C. *A. granosa* grown for 64 days. Slowing down of shell growth began at about day 44th (numbered arrow) onwards.

D. *A. antiquata* grown for 118 days, showing the dominant structure of complex crossed lamellar layer which obscures the appearance and detail of the microgrowth bands.

E. *A. granosa* grown for 64 days, showing 64 widely spaced growth bands. The numbered arrows indicate the growth bands deposited during the period of maximum spring tides (S).

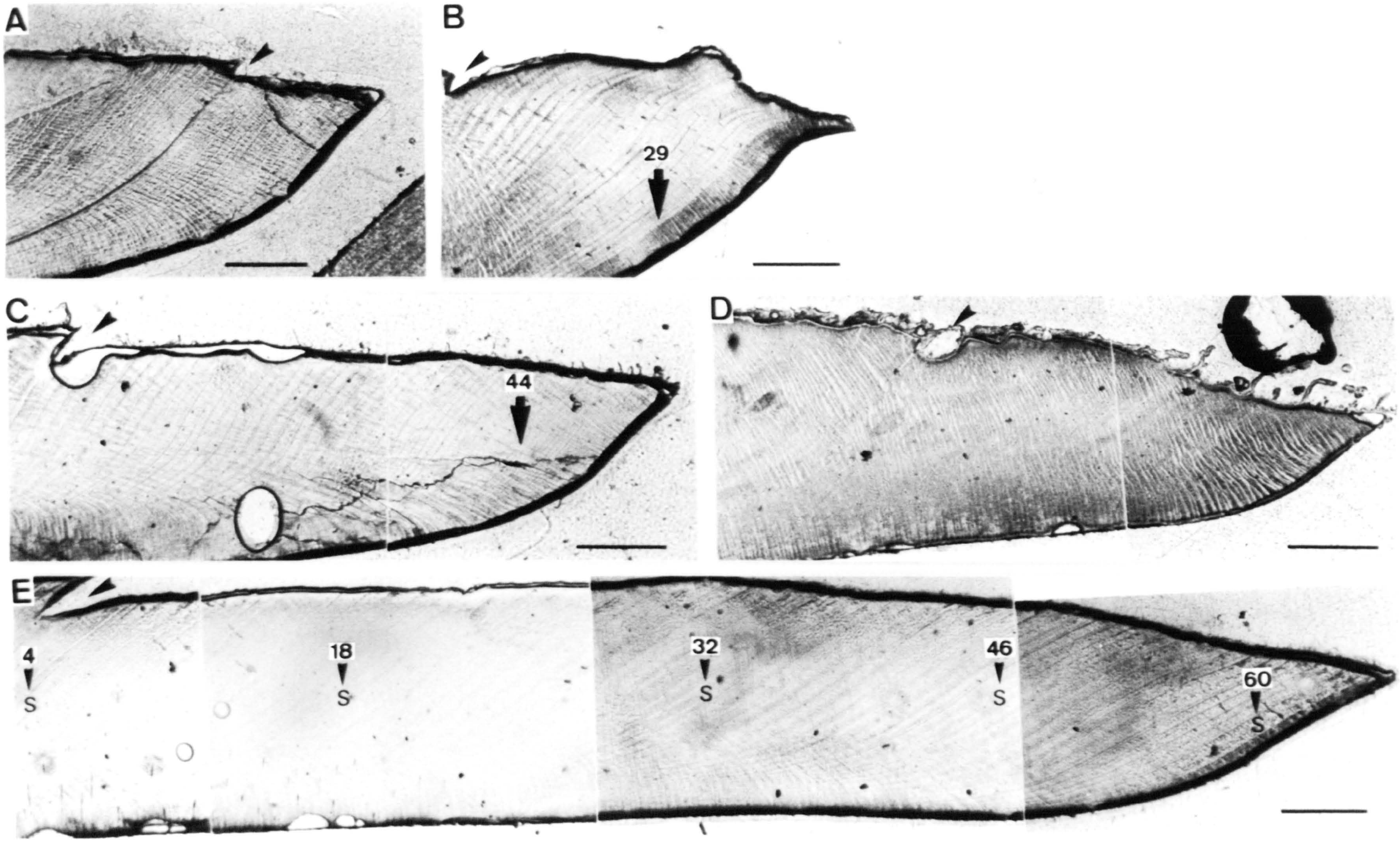
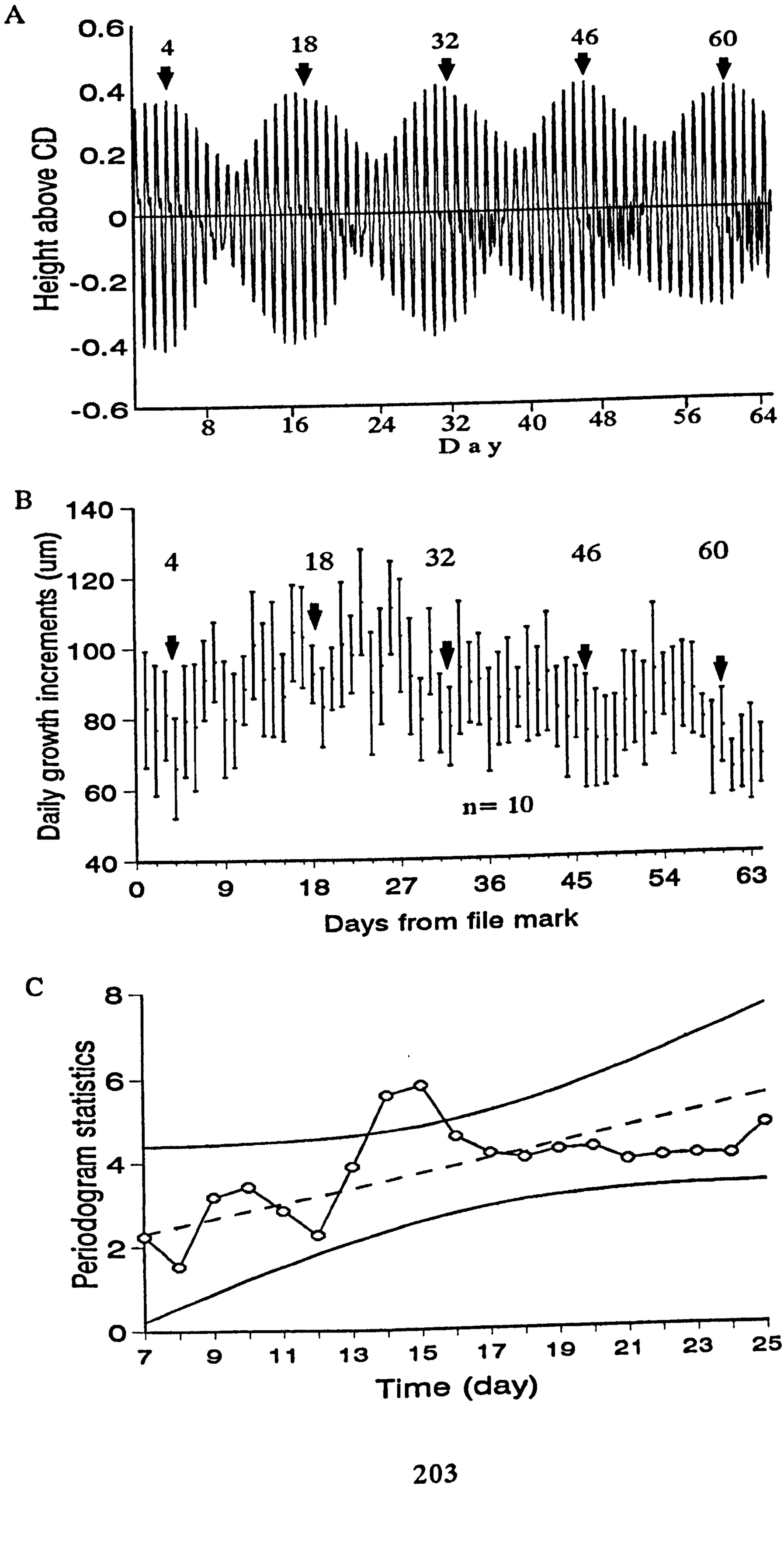

A
B
29
C
44
D
E
4
S
18
S
32
S
46
S
60
S

Figure 34:

A. A predicted tidal model for Semarang waters during the 64 days period of the experiment, 5 June-8 August 1993. The day when the maximum spring tides occur are arrowed and numbered.

B. Variation in the width of the daily growth increments of file-marked *A. granosa* transplanted for 64 days in the field at Wedung. Increment widths of 10 clams of size 16.8mm to 25.6mm which were not reproductively active during the 64 days of the experiment are shown. The narrowest growth increments coincide with or are deposited a few days after the maximum spring tides which are arrowed and numbered.

C. Periodogram analysis for time series data shown in B, showing a significant spring-neap lunar cycle occurring with a periodicity of 14-15 days.


A
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Height above CD
4
18
32
46
60
8
16
24
32
40
48
56
64
Day
B
140
120
100
80
60
40
Daily growth increments (um)
4
18
32
46
60
n= 10
0
9
18
27
36
45
54
63
Days from file mark
C
8
6
4
2
0
Periodogram statistics
7
9
11
13
15
17
19
21
23
25
Time (day)

From the histological study of the reproductive tissue in Chapter III, it is clear that in July 1992 the clams were ripe and some were starting to spawn. By August the following month, there was conclusive evidence of a distinct spawning. Plate 8C shows part of a peel from a shell of 19.6mm showing a group of narrowly spaced increments (large arrow) alternating with a group of widely spaced increments (small arrow) and another group of narrow increments (large arrow). Similarly, an examination of the acetate peels of large *A. granosa* (53.6mm) clearly showed a seasonal variation in the width of the growth increments (Plate 8D). Generally the pattern consisted of wide increments alternating with areas in which narrow increments were deposited. Furthermore, gonad index is related positively to the condition index ($r= 0.351$; Table 11 in Chapter III), whereas condition index is always negatively correlated with shell growth increment ($r= -0.210$; Table 20) although neither of them are statistically significant. The correlation between gonad indices and shell deposition on the other hand is always positive, particularly for male clams ($r= 0.688$; $P_{0.02,df23}= 0.620$; Table 20). This means that whilst clams are reproductively more active, somatic growth, in terms of the rate of shell deposition, is reduced.

In order to investigate whether there was a seasonal variation and which factors were responsible for influencing the width of the microgrowth patterns, the width of the last 14 increments, (≈14 days, or equivalent to one spring-neap lunar cycle, hitherto known as a semilunar tidal cycle), deposited at the shell margin was measured in approximately 4-10 blood clams collected each month. Figure 35A shows the seasonal variation in the average width of the semilunar growth increments measured from the width of the last 14 days deposited increments taken from each of the monthly samples. Two trends are apparent; minimum growth during February-March 1992 and 1993, September 1992, and June-August 1993, maximum growth was

between May and June 1992 as well as 1993. Furthermore, there is no apparent relationship when comparing the seasonal variation in shell growth to the temperature, oxygen saturation content, salinity and chlorophyll-a concentration. The non-significant and significant correlation coefficients (r) are listed in Table 20. Only shell growth and the amount of rainfall showed a significantly negative correlation (r= -0.466). Figures 35 compares the seasonal variation in the semilunar growth increments (A) to the monthly rainfall, salinity and sediment temperature (B to D).

A further search for other possible factors which might influence the seasonal variation in shell growth revealed that the reproductive condition of the blood clams was a factor in controlling shell growth. When the seasonal pattern of male, female and population gonad indices previously established in Chapter III, are plotted with the seasonal variation in shell growth, there is a positive correlation (Table 20, Fig. 36) between the two factors. On six out of seven occassions between 1991-1993 when there was a decrease in the mean male gonad index (Fig. 36A) there was a similar decrease in shell growth rate. A significant and positive correlation ($r= 0.688$; $P<0.002$, Table 20) between these two factors exists. Whereas when the female and population gonad indices were compared with the shell growth rate, although there were positive correlations between the two variables, these were not significant (Figs. 36B and C and Table 20). Histological studies in Chapter III showed that July-September 1992 and June-August 1993 were the major spawning seasons for *A. granosa* in Wedung and these periods coincided with lower shell growth rates, particularly during 1993 (Fig. 35A). Periods of gamete development in February-March 1992 and 1993 when both female and male clams showed a decrease in the gonad index also coincided with a decrease in width of the shell growth increments.

Spawning which was only observed in females as a decrease in gonad

Table 20: The Spearman rank order correlation between microgrowth increments within the shell of *A. granosa* from natural population in Wedung and some environmental as well as physiological parameters

Parameters	**Shell Microgrowth Increment**
Water temperature	-0.061 ns
Sediment temperature	-0.189 ns
Oxygen saturation value	-0.295 ns
Monthly rainfall	-0.446 *
Chlorophyll-a	-0.343 ns
S a l i n i t y	-0.176 ns
Condition Index	-0.210 ns
Male Gonad Index	0.688 **
Female Gonad Index	0.209 ns
Population Gonad Index	0.317 ns
Population Dry Tissue Weight	-0.021ns

Degree of significance (df= 23): ns= not significant; * P<0.05= 0.417;
** P<0.002= 0.620.

Figure 35: Seasonal variation in the average width of the semilunar growth increments of *A. granosa* from Wedung and its relationship with some environmental factors.

A. Seasonal variation in the average width of the semilunar growth increments showing minimum growth in February-March 1992/1993, August 1993 and maximum growth between May-June. The number of blood clams examined each month is given above the 95% confidence interval.

B-D. A comparison of the seasonal variation in shell growth with (B) the amount of monthly rainfall, (C) salinity and (D) sediment temperature .

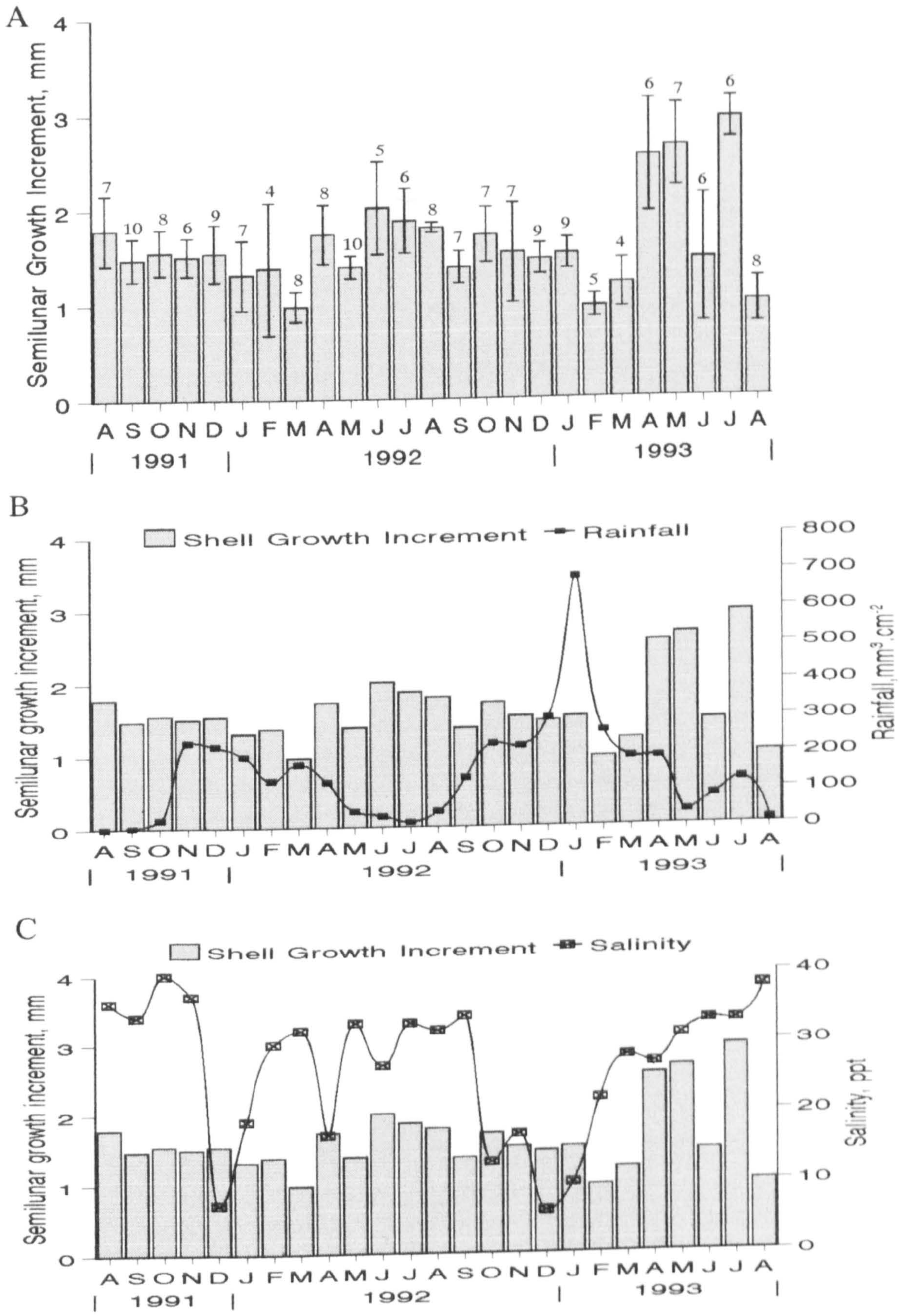

A
Semilunar Growth Increment, mm
0
1
2
3
4
7 10 8 6 9 7 4 8 8 10 5 6 8 7 7 7 9 9 5 4 6 7 6 6 8
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
B
Shell Growth Increment
Rainfall
Semilunar growth increment, mm
Rainfall, mm³.cm⁻²
0
100
200
300
400
500
600
700
800
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
C
Shell Growth Increment
Salinity
Semilunar growth increment, mm
Salinity, ppt
0
10
20
30
40
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993
D
Shell Growth Increment
Temperature
Semilunar growth increment mm
Sediment temperature, °C
20
25
30
35
40
A S O N D J F M A M J J A S O N D J F M A M J J A
1991
1992
1993

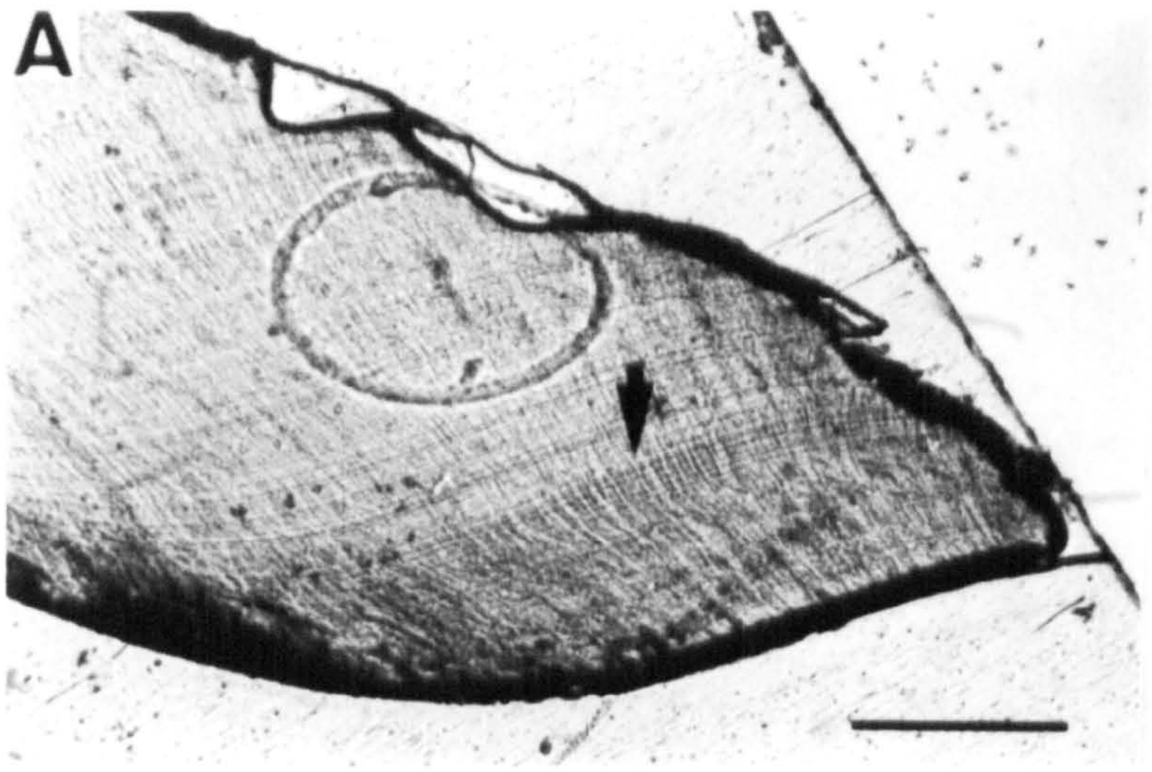
Plate 8: Photomicrographs of acetate peels from shell sections of *A. granosa* selected from the monthly samples. Growth from left to right. Scale bars = 500μm, except D = 1mm.

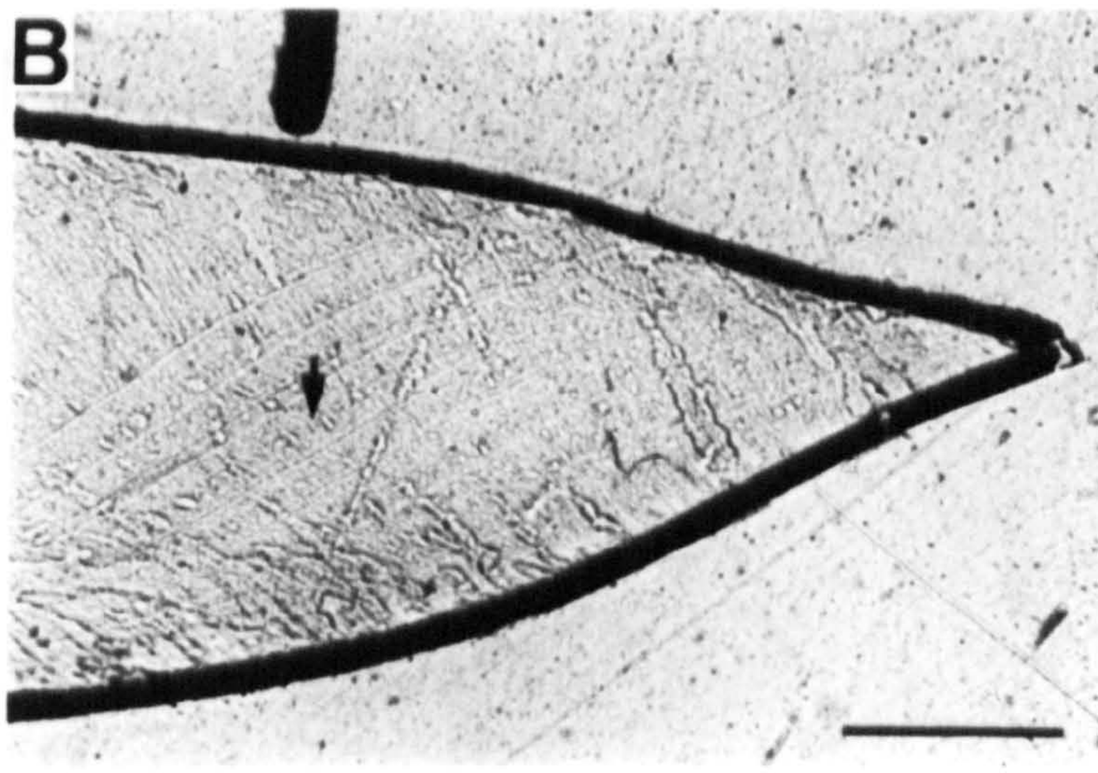
A. *A. granosa* of 20.4mm shell length collected in August 1992, showing narrow growth increments at the shell margin.

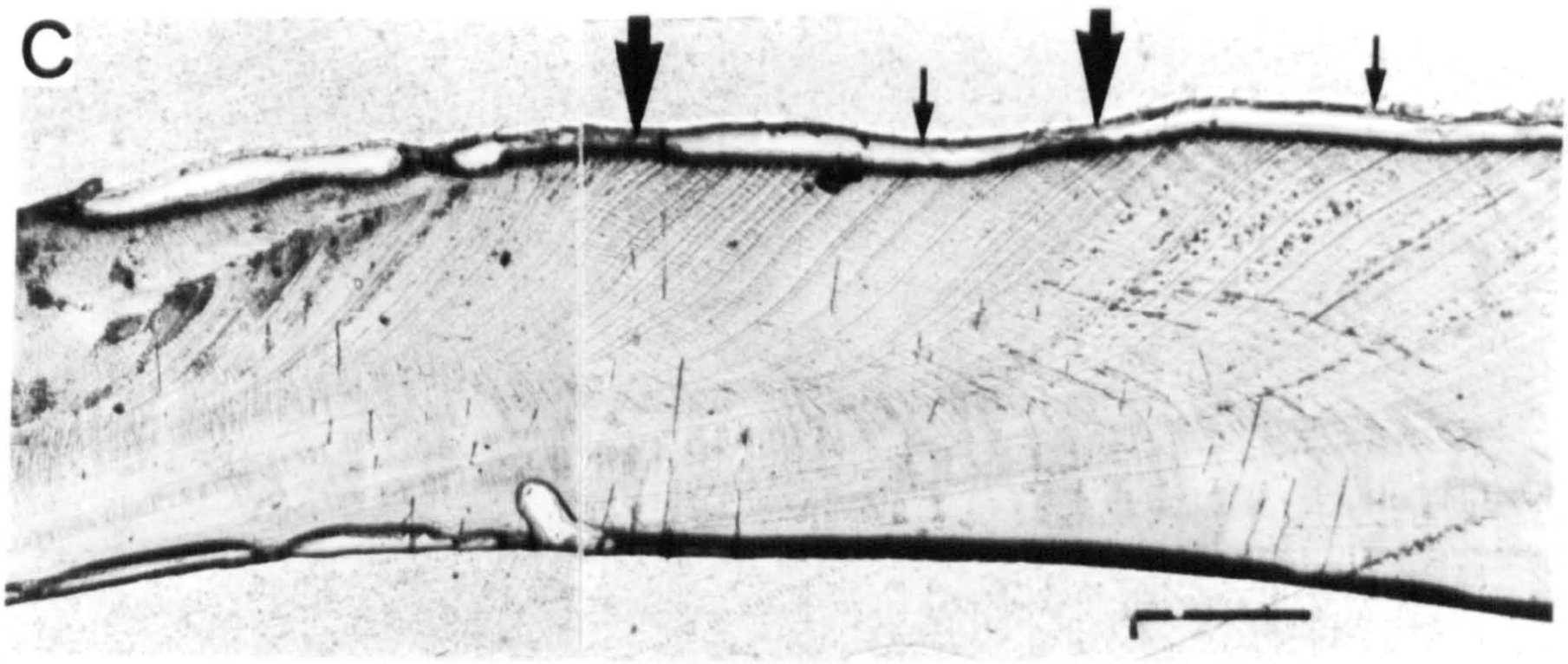
B. *A. granosa* of 25.1mm shell length collected in July 1992, showing wide increments of bands at the shell margin.

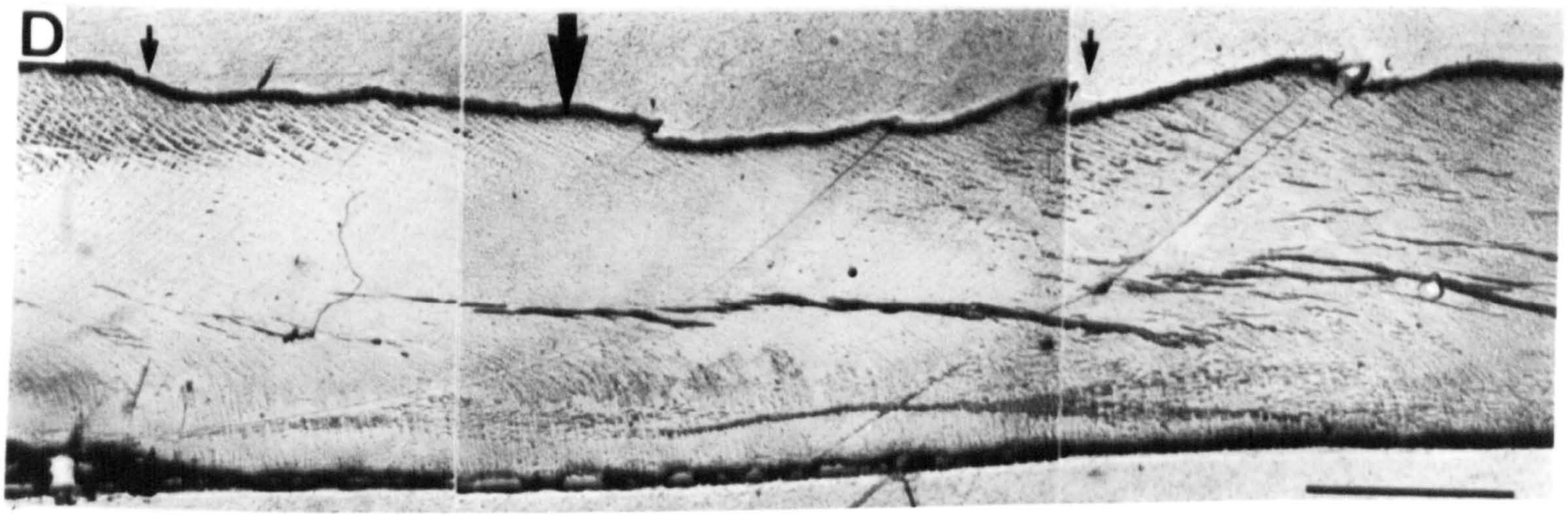
C. Part of a peel of an *A. granosa* of 19.6mm length, showing alternating periods of narrow (large arrow) and wide increments (small arrow) .

D. Part of a peel of *A. granosa*, showing alternating periods of narrow (large arrow) and wide increments (small arrow) from a large individual (53.6mm).


A


B


C


D

index also occurred in October-December 1991, and this did not affect the width of the shell growth increments (see Fig. 36C). The lack of correlation in females between shell growth and reproduction is however not surprising, because shell sections prepared for microgrowth increment analysis were randomly taken from 15-27mm individuals consisting only of males. Thus it has been seen that the seasonal pattern of shell growth is affected by period of gamete development and spawning and that acetate peels of both experimental and natural populations of *A. granosa* shells showed evidence of a decrease in the width of growth increments during spawning. Since there are two spawning periods in the year, i.e. around March and August, there will be two groups of narrow growth increments deposited each year in the shell as seen in Plate 8C.

Having established that a seasonal pattern of narrowing of the growth increments was present in the shells of *A. granosa*, 14 of the largest blood clams from Wedung and Tapak were selected and acetate peels prepared. The positions where the bands narrowed in the peels were marked along the shell on the coverslip using a fine drawing pen, and the distance between the umbo and successive marks along the surface of maximum growth of the shell measured using a calibrated eye piece micrometer. The cumulative measurements of this radial axis (p) were then converted into transversal axis measurements (shell length, SL) using the simple regression equation **p= 1.46+ 1SL** determined previously. The von Bertalanffy growth equation (VBG) was then fitted to the length at age data and values of L∞ and K determined using the software package FISHPARM.

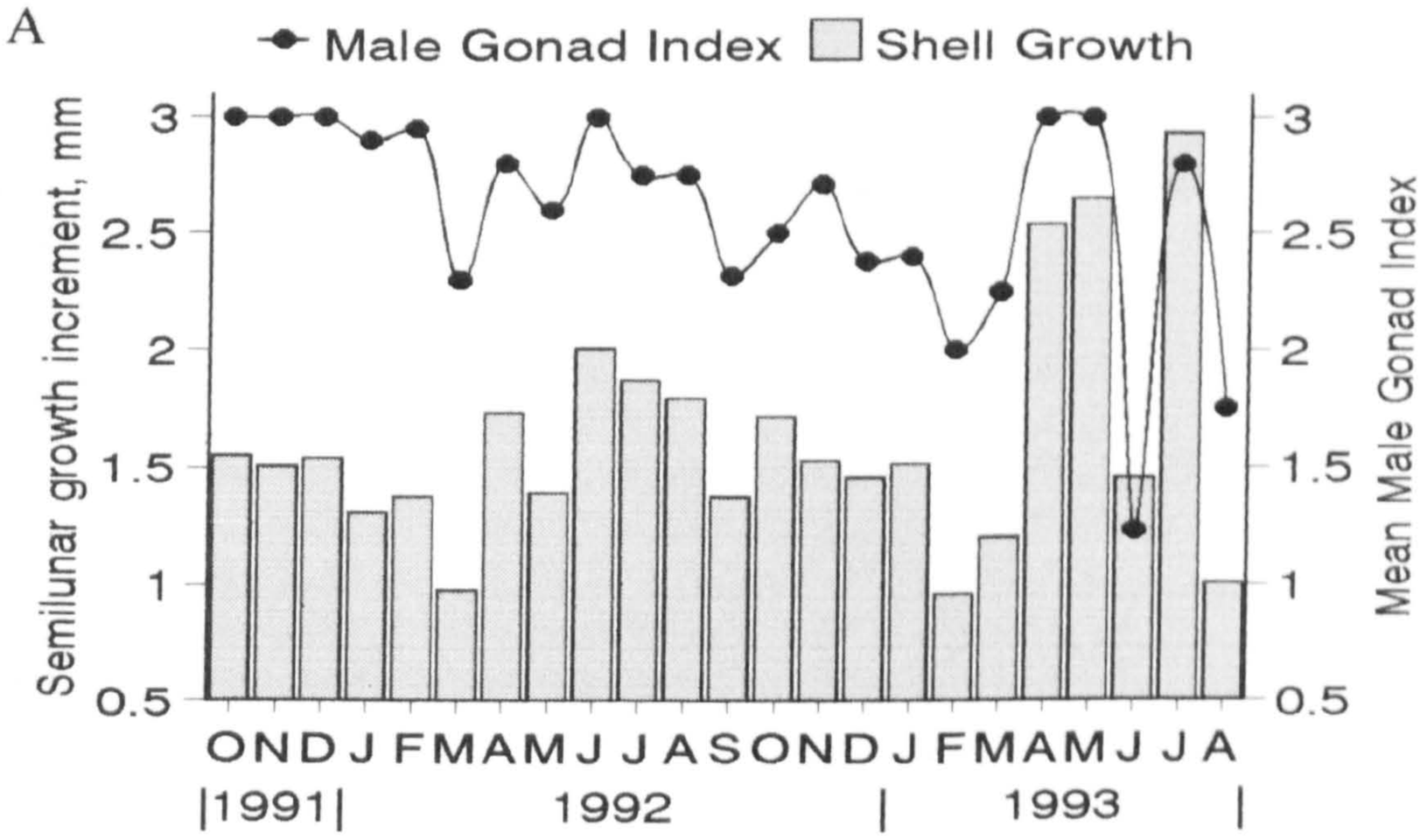
The growth curves of *A. granosa* generated from the von Bertalanffy equation for those of the largest specimens within the populations at Wedung and Tapak are shown in Figure 37A. Two features emerge, firstly the maximum attainable size reached by both groups of blood clams is similar,

Figure 36: Seasonal variation in the width of the semilunar growth increments of *A. granosa* and the average gonad index determined from the monthly samples.

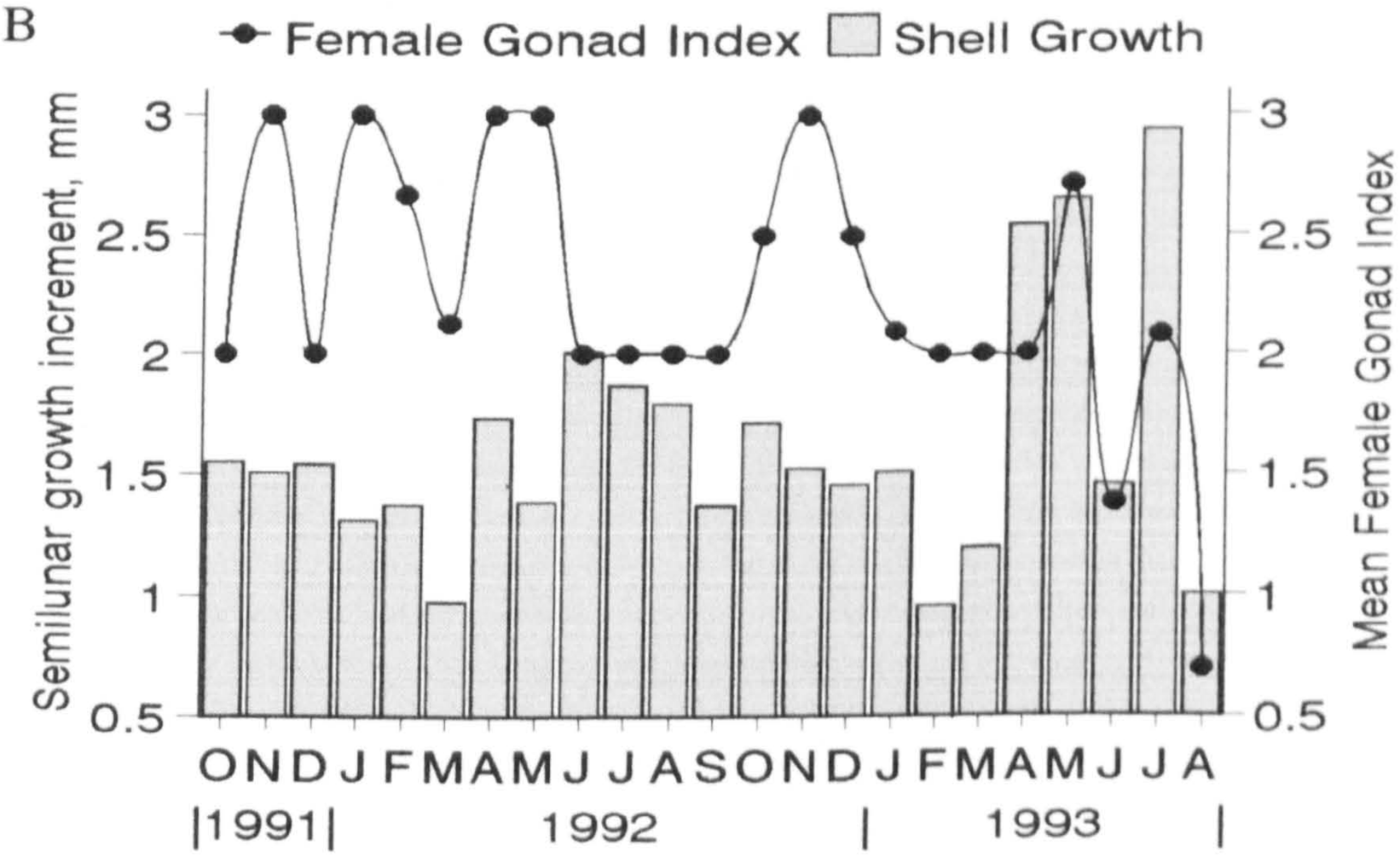
A. Male blood clams
B. Female blood clams
C. Male and female combined.

The correlation between shell growth and gonad index is significant only for male blood clams ($r = 0.688$, $P<0.02$).

A


Male Gonad Index
Shell Growth
Semilunar growth increment, mm
Mean Male Gonad Index
3
2.5
2
1.5
1
0.5
O N D J F M A M J J A S O N D J F M A M J J A
|1991|
1992
|
1993
|

B


Female Gonad Index
Shell Growth
Semilunar growth increment, mm
Mean Female Gonad Index
3
2.5
2
1.5
1
0.5
O N D J F M A M J J A S O N D J F M A M J J A
|1991|
1992
|
1993
|

C

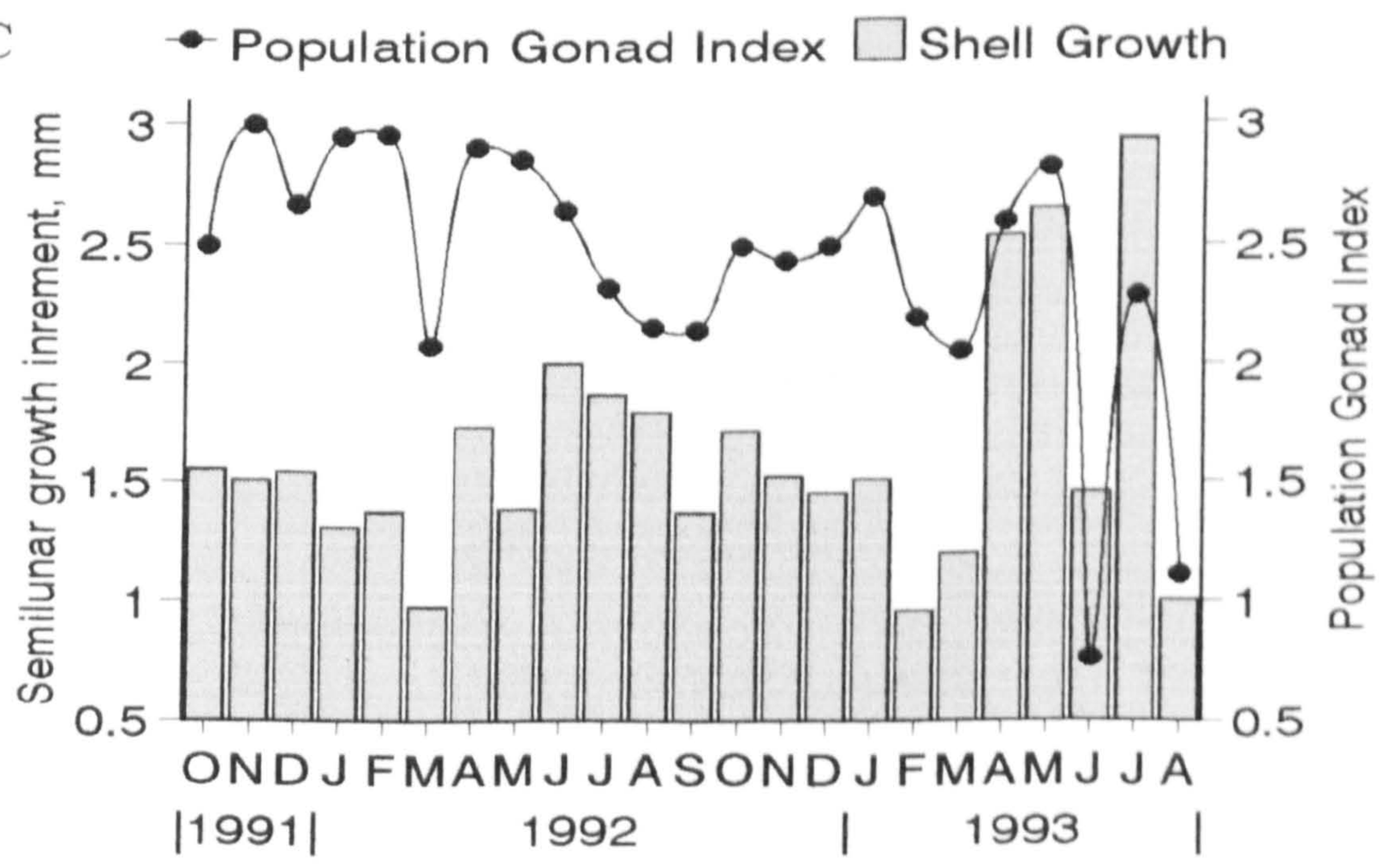
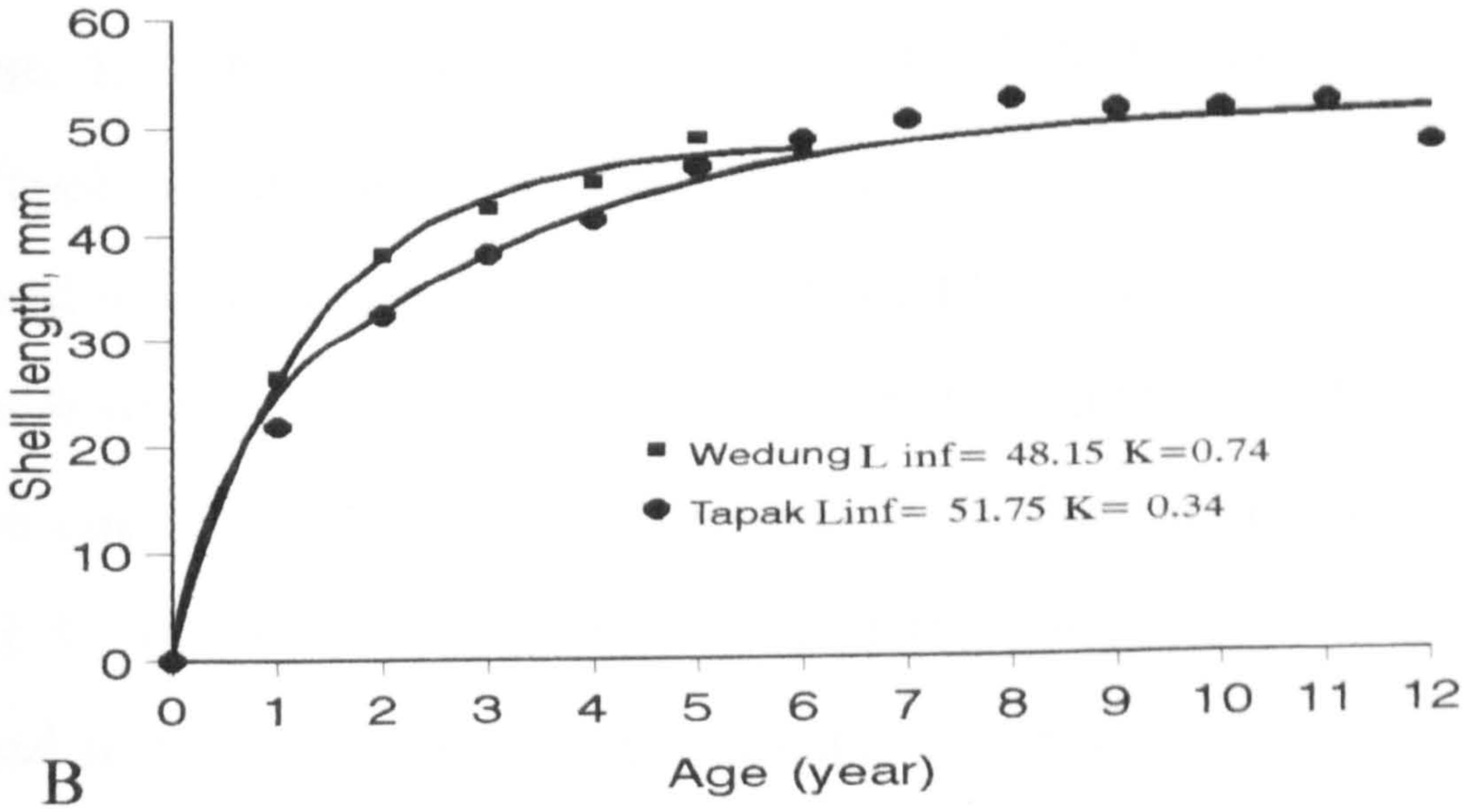

Population Gonad Index
Shell Growth
Semilunar growth inrement, mm
Population Gonad Index
3
2.5
2
1.5
1
0.5
O N D J F M A M J J A S O N D J F M A M J J A
|1991|
1992
|
1993
|

Figure 37: Growth curves and size frequency distributions of *A. granosa*

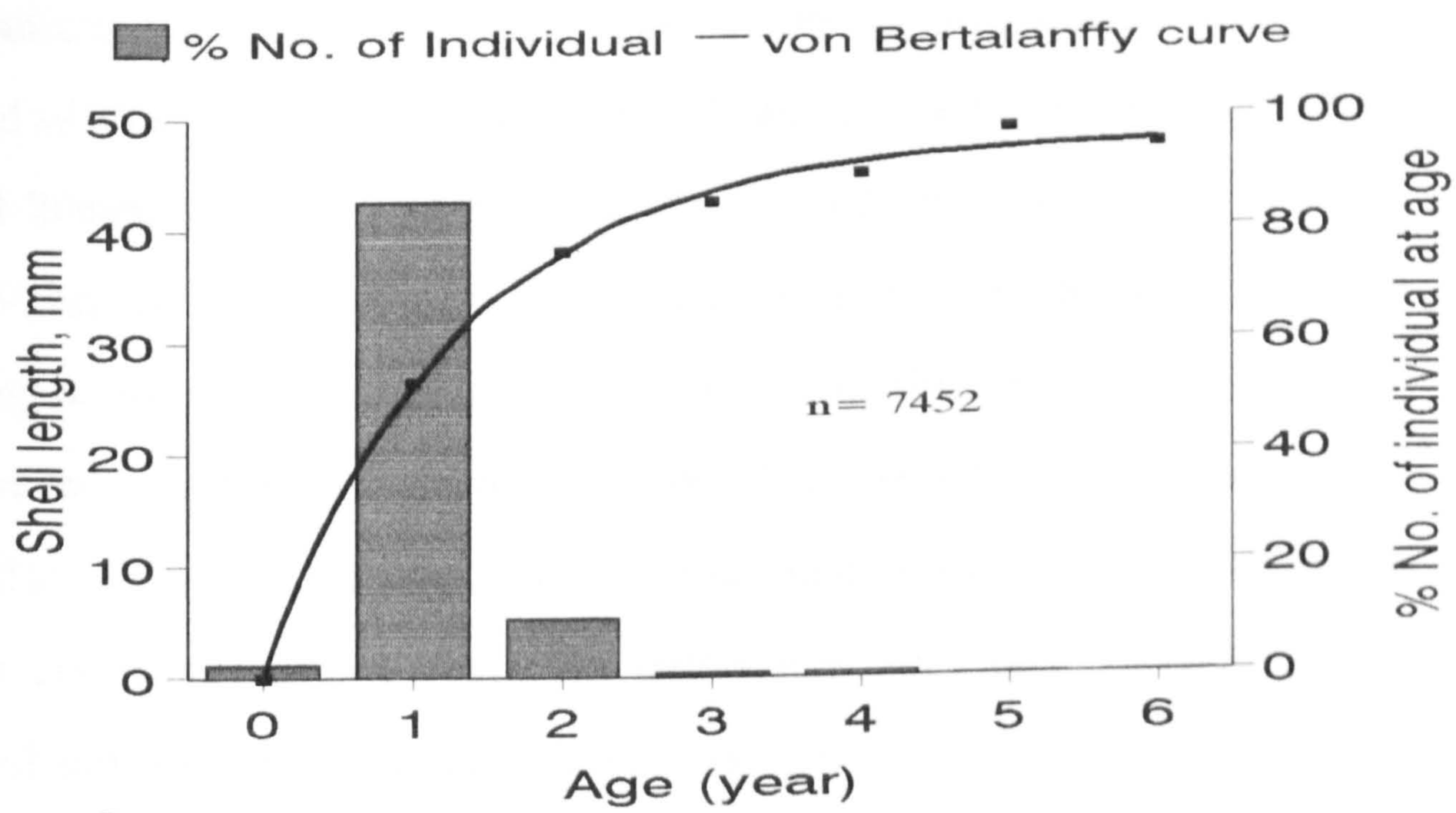
A. Growth curves for the population in Wedung and Tapak. The von Bertalanffy growth equation has been fitted to the data obtained from the microgrowth patterns in the shell.

B-C. A comparison between the size distribution and age of *A. granosa* from B) Wedung and C) Tapak.

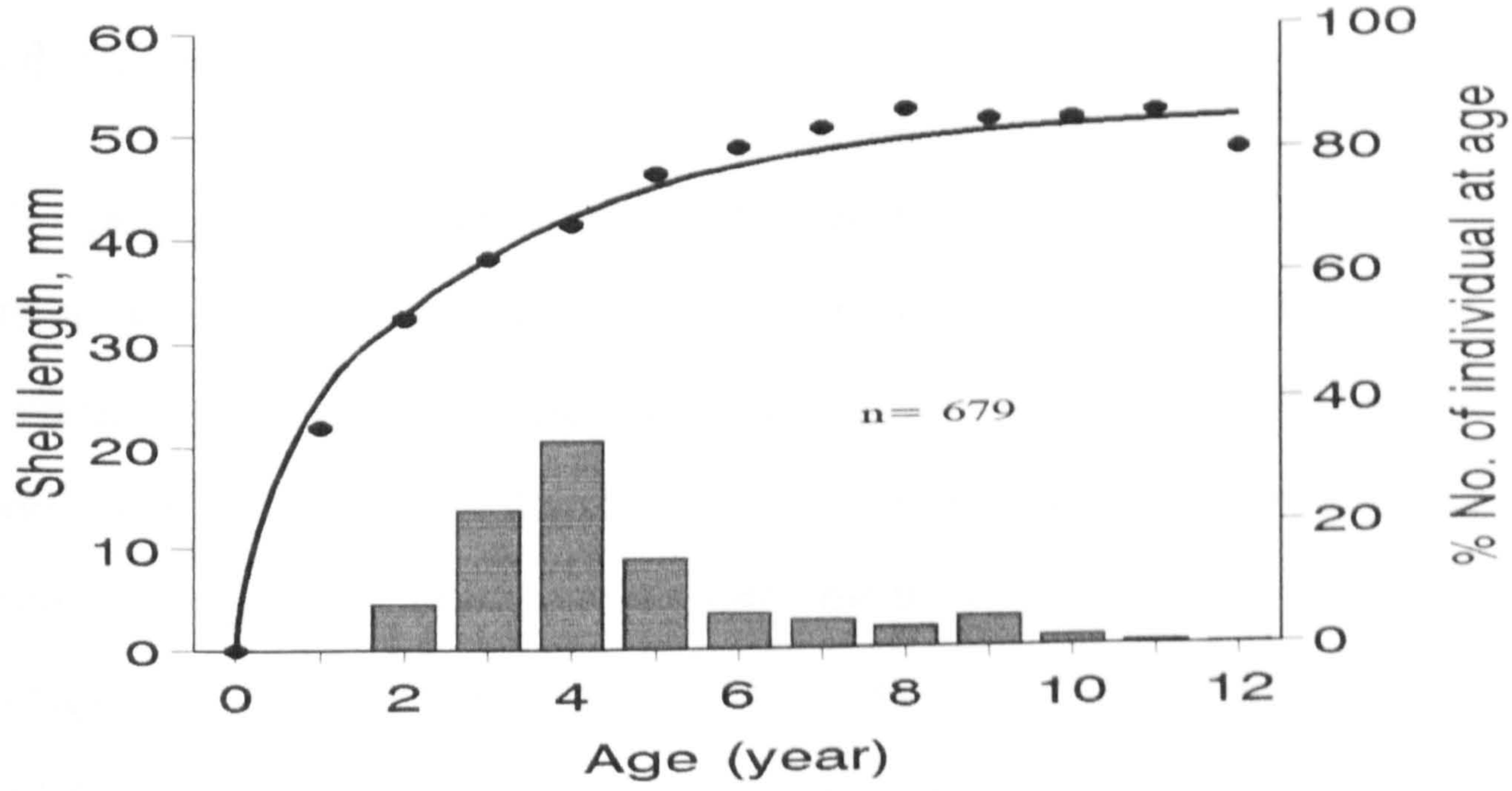
A


Shell length, mm
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Age (year)
Wedung L inf= 48.15 K=0.74
Tapak Linf= 51.75 K= 0.34

B


% No. of Individual
von Bertalanffy curve
Shell length, mm
50
40
30
20
10
0
0 1 2 3 4 5 6
Age (year)
n= 7452
100
80
60
40
20
0
% No. of individual at age

C


Shell length, mm
60
50
40
30
20
10
0
0 2 4 6 8 10 12
Age (year)
n= 679
100
80
60
40
20
0
% No. of individual at age

L_{∞}= 48.15mm and 51.75mm for Wedung and Tapak respectively, but the growth rate K is dissimilar with a value of K= 0.78 for Wedung and 0.34 for the Tapak population (Fig. 37A). Secondly, *A. granosa* from Tapak are longer lived, reaching an age of 13 years whilst those from Wedung achieve only an age of 6 years. It can be seen from the growth curves (Fig. 37A) that blood cockles reach a size of between 22 and 26mm during their first year, and 33-38mm in their second year. These sizes are similar to those determined from the modal size progression in the monthly size frequency distributions (see Fig. 29). Separation of modal size classes using the method of Bhattacharya has shown that *A. granosa* achieve an approximate size of 20mm in the first year and by the second year are nearly 40mm in size. When the age distribution of blood clams in the population at Wedung and Tapak are compared (Figs. 37B to C), it is obvious that the Wedung population is composed mainly of individuals less than 2 years of age (85.28%) whereas the majority of the population in Tapak (34.32%) are mostly comprised of 3-4 years old individuals with up to 35.5% of the blood clams comprising the age classes 5-12 years.

4.4. Discussion

The only published study which described some information on the allometric growth in *A. granosa* was carried out by Narasimham (1988). Some allometric relationships taken from that study are comparable to those measured in the present investigation, e.g. the relationships between height (assumed as height 2 in this study), width, hinge plate and shell weight to shell length as the independent variable. The author however, did not evaluate whether there was a significant departure of those coefficients of allometry from isometry. However they were all presumably negatively allometric, as their growth exponent (b) lay well below the isometric value

Table 21: Comparison between several allometric relationships in *A. granosa* from India (Narasimham, 1988) and from Indonesia waters (rounded morph from Wedung, present study)

Variable		Coefficient of allometry of *A. granosa* from:					
		India			Indonesia		
Dependent	Independent	a	b	Allometry	a	b	Allometry
Height 2	Length	1.018	0.785	negative	0.8872	0.957	isometric
Width	Length	0.971	0.710	negative	0.5875	1.050	positive
Hinge Plate	Length	1.022	0.742	negative	0.5382	1.090	positive
Shell Weight	Length	-2.967	2.595	negative	0.0009	2.600	negative

(Table 21). These negative relationships indicate that in Kakinada Bay, India, blood clams, *A. granosa*, grow faster along the transversal axis to attain a larger size, whilst in Wedung and Tapak, their lateral expansion was more pronounced than any other linear dimensions of growth. These differences show how much variation there is in the shell shape amongst populations which is due simply to changes in shape with size. A comparison with the study of Patel and Patel (1974) was not possible because their measurements were not intended to study the allometric relationships in *A. granosa*. The authors used simple regression equation of linear rather than log transformed data in the allometric growth equation. When shell weight and length are compared, e.g. in Table 21, it is clear that data for *A. granosa* from India and Indonesia are similar (b= 2.595 and 2.600 respectively). For species living on very soft sediments into which they would readily sink, heavy shells could be a disadvantage. Thayer (1975) for example, found that the growth exponent (b) relating shell weight and length in two mud-dwelling bivalve species *Mulinia* (b= 2.75±0.82) and *Nucula* (b= 2.57±0.52) was significantly less than 3, indicating that in these species larger shells would be lighter per unit length. On the other hand, enhancing physical stability by increasing bulk density could be advantageous to those species that recline freely on the sediment surface or to shallow burrowers that are subject to frequent wash-out by currents (Seed, 1980). The shells of the congener, *A. antiquata*, which is a shallow burrower living in a sandy intertidal habitat presumably provides a better resistance and support. The shell weight-length relationships in this species were also significantly negatively allometric although the b value (2.79) is somewhat higher than that observed in the rounded form of *A. granosa* (b= 2.60 and 2.67, Table 15) from Wedung and Tapak respectively.

The use of size frequency distributions is usually suitable only for species with distinct seasonal recruitment and relatively uniform growth rates

amongst individuals in each year class (Cerrato, 1980; Anwar et al, 1990a). As shown in Chapter III, the two populations of *A. granosa* studied developed their gametes in February-March and spawned in the period of July-August but this was also followed by a low level of spawning activity throughout the year. Even though there were only three distinct spatfalls (during July-August), low recruitment of spat may occur throughout the year. Size frequency distributions therefore have limited application in estimating growth rates of *A. granosa*, as an inevitable merging of size classes may result. Nevertheless, in this study the method of Bhattacharya applied to *A. granosa* from the Wedung population distinguished at least three major recruitments throughout August 1991 to August 1993.

Surface growth checks or rings, the method usually used to determine the age of bivalves were found to be of limited use in age determination of this particular species as they were rarely discernible on the shell surface (Narasimham, 1969; Richardson, 1987; Anwar et al, 1990a). However, some previous studies, particularly on *Anadara* (= *Senilia*) *senilis*, another species of anadarinid, have utilised surface growth rings as annual marks in determining the age of individuals (Okera, 1976; Wolff et al, 1987).

Species like *A. granosa* and *A. antiquata* produced planktotrophic larvae. Such larvae spend long periods in the plankton (>3 months) compared to lecithotrophic ones (<3months; Sastry, 1979). However, Wong et al (1985) have recently reported that in the laboratory, metamorphosis of the larvae of *A. granosa* into juveniles in is usually complete within 26-28 days. *A. antiquata* from the Bandengan population has a more seasonal reproductive cycle, although nothing is known about the duration of their post-larval stages. The application of Bhattacharya's method for separation of the size frequency distribution of this species identified four separate cohorts although it was not possible to identify the particular month of recruitment.

One possible reason for this is may be due to the sampling technique used which was unsuccessful in collecting spat smaller than 10mm in diameter, so that it was only possible to construct length frequency histograms from animals larger than this size.

Assuming that *A. antiquata* leads a prolonged pelagic larval life, the period over which they recruit into the population might also occur over a long period of several months as has been shown in the limpet *Patelloida pygmaea* (i.e. 8 months, Liu, 1994). If this is the case, then size frequency distributions are of little use in describing the population growth rate of *A. antiquata*. This was because even though spawning in this species is more seasonal than its congener *A. granosa*, the method is suitable only for species with distinct seasonal recruitment and relatively uniform growth rates among individuals in each year class.

Somehow, none of the previous workers (e.g. Kastoro, 1978; Toral-Barza & Gomez, 1985; Kayombo & Mainoya, 1987; Kasigwa & Mahika, 1991) studied specimens of ≤10mm in length nor attempted to identify the time of recruitments for *A. antiquata*, and there was a considerable overlap in the average size and range of the specimen examined; i.e. 38.5-50.7mm (Toral-Barza & Gomez, 1985), 33 and 43mm (Kayombo and Mainoya, 1987), 33.8mm (Kasigwa and Mahika, 1991), and 33mm in the present study. Kayombo and Mainoya (1987) were able to derive growth rates of *A. antiquata* from the modal progression analysis and gave an estimate of 1.7mm.month^{-1}. Using a similar approach in this study, the overall rate of growth of *A. antiquata* in Bandengan was much slower, i.e. 1mm.month^{-1}.

From a histological study of the reproductive cycle of *A. antiquata*, it is clear that they reached a size larger than that of *A. granosa* before they began to undergo gonad development (Chapter III). Continued growth for several years before reproduction, has been noted in the horse mussel *Modiolus*

modiolus and is thought to be a strategy for escaping predation (Seed & Brown, 1978). *A. antiquata* may also employ this tactic, growing to a size large enough to escape predation from mobile epibenthic species particularly large size brachyuran decapods (see Chapter II), before then undergoing reproductive development.

Irrespective of the variation within the monthly samples as to the time of collection, the average monthly growth rates calculated from the acetate peels is about 2.51mm.month^{-1} during the six months between August and January 1992 and 2.67mm.month^{-1} from August 1992 to January 1993 respectively. The modal progression analyses gave an average size increment of 2.3mm. month^{-1} in August 1991-January 1992 and 3.3mm.month^{-1} during six months in August 1992-January 1993 (Figs. 29, 30A). This average shell growth determined from shell sections during August 1992-January 1993, i.e. 2.67mm.month^{-1} is somewhat higher than the findings of the modal progression analysis, 3.3mm.month^{-1}. The likely cause of this discrepancy in the monthly growth rate between August 1992 and January 1993 calculated by these two different methods, was perhaps due to the activities of commercial harvesting that may have altered modal size of the length frequency histogram by collecting all available size classes of blood clams from the populations.

Wong et al (1985) observed that *A. granosa* attained shell lengths of 2.5mm within 28 days after fertilisation. Narasimham (1969) observed that in natural beds in Kakinada Bay, India, spat of *A. granosa* of 4.5mm showed an increase in size of 3mm in a period of two months. However, Broom (1982b, 1985) reported that natural populations of *A. granosa* in West Malaysia take approximately six months to grow to 4-5mm length. This rate was very much lower than that reported from other studies.

Assuming the larvae spend 28 days in the plankton (Wong et al, 1985) and that blood clams in this study grow at an average rate of 2.51mm.month^{-1}. Animals of 14-16mm in size recruited into the population in July and August 1992 and 1993 would presumably have resulted from a spawning in the previous November and December. Whereas those recruits of 22-24mm in March 1992 and 1993, assuming similar rates of growth, would have arisen from a spawning in April or May 1991-1992 respectively. The pattern of growth increments widths broadly follows the cycle of development of the gonad observed in Chapter III. Similarly Broom (1982b, 1983b), using back-extrapolation of growth curves combined with histological examination, reported that a spatfall of *A. granosa* in West Malaysia appeared in June 1979 following a major spawning in late December 1978-February 1979 which coincides with a pronounced salinity depression.

In this study, measurement of salinity at the three sites indicated that the lowest values occurred during December (Figs. 7-9 Chapter II). A similar finding was found by Broom (1982b). It has been shown in this study that shell growth is significantly and negatively correlated to the amount of rainfall (see Table 20), which means that during the rainy season there will be a shell growth cessation. Accordingly, it has been shown that water and sediment temperatures become significantly warmer during the rainy season, and that the warmer temperatures combined with low salinity and abundant food coincide with one of the periods when *A. granosa* spawns.

A comparison of the seasonal variation in the semilunar growth patterns and mean male gonad index show there is a significant positive correlation. From this finding it is suggested that during major reproductive periods (March to August 1992 and February to August 1993) when the gametes in the gonads are developing to the ripe stages, the shell growth slows down for

a while as evidenced by a narrowing of the growth increments until the gametes are discharged (see Fig 36C). This pattern was observed both in the experimental animals and those *A. granosa* collected in the monthly samples. Broom (1982b), did not attempt to correlate shell growth to the reproductive conditions. The findings of this study and those of Richardson (1987) found a reduction in shell growth of *A. granosa* in Penang, Malaysia during periods of heavy rainfall when the salinity was lowered.

The growth patterns observed in *A. granosa* were generally well defined whereas in *A. antiquata* they were very difficult to interpret and resolve. Taylor et al (1969) stated that the pattern in *A. antiquata* is very different and much more subdued in comparison to most other species of Arcaceae, Limopsacea, Pectenacea and Limacea they had examined. Moreover, the complexity of the pattern is comparable only to *Barbatia fusca.*

During spring tides experimental *A. granosa* growing in the shallow subtidal would remain covered at low tide by a shallow depth of water (20-30 cm) and during neap tides they would experience little change in water depth. Figure 34 showed that the pattern of bands observed in the shells of *A. granosa* reflected a semilunar cycle, the growth of the shell becoming significantly reduced during or a few days after maximum spring tides.

The pattern of reduced shell growth during spring tides and enhanced growth at neap tides is comparable to that observed in the temperate water common cockle, *Cerastoderma edule,* living near low water. They grew faster during the neap periods when the shells were continuously immersed (Richardson et al, 1980b). Furthermore the authors demonstrated that the reverse held for animals grown at mean high water of neap tides (MHWN). The cockles growing significantly more during spring tides when they were immersed for longer periods for feeding than during neap tides, i.e.

when they were emersed for all but a few minutes of each tidal cycle. In the present study, the experimental *A. granosa* were never completely exposed to air, but presumably the periodic daily lowering of the water surface and possibly water temperature changes during the ebb and flood tide provided the stimulus for growth band formation.

In a previous study of *A. granosa* in Penang, Malaysia (Richardson, 1987), blood clams were experimentally marked and grown in intertidal and subtidal areas. The tidal prediction for Penang has a mixed semidiurnal pattern with two highs and two lows of unequal amplitude each day, so that the number of tides is almost double the number of days. Deposition of microgrowth increments in the majority of blood clams corresponded to the number of tidal periods. Furthermore, the banding pattern in subtidal shells showed narrow increments during spring tides alternating with a few wider increments during neap tides. This finding is in agreement with the results of the present study. Plate 7E shows a peel from a marked blood clam grown for 64 days on the low shore where they would experience near-emersion during spring tides, and this clam displayed a similar banding pattern to that observed in Figure 8 of Richardson (1987).

In estimating the age of individual clams Richardson (1987) counted the number of microgrowth bands within a given distance, i.e. at regular 1mm intervals along the surface of maximum growth and a polynomial equation was applied to give the best fit to a plot of number of bands.mm^{-1} and shell length. Richardson (1987) showed there was a periodic narrowing of growth increments and distinct growth lines which coincided with the annual periodicity of lowered salinity during the monsoon. These lines were then used to estimate the annual growth rate of the blood clam. In this study the periodic narrowing of growth increments during the spawning season were

used to estimate annual growth rates and growth curves plotted from these data.

Although the method used by Richardson (1987) gives an accurate range of age (in days), the disadvantage of the method is that the growth record during the early part of the clam's life may not be present because the outer shell layer in the umbo region was usually severely abraded in the blood clams from Penang, in particular for large individuals. The method used in this study was found more convenient, simple and applicable for larger sample size, once the periodicity of formation of the microgrowth bands had been documented.

Richardson (1987) was able to demonstrate from back calculation of the growth patterns in *A. granosa* from Penang that it was during periods of low salinity in the monsoon that distinct dark lines were deposited in the shell of a few blood clams. However, in this study the width of the semilunar growth increments were not appreciably reduced after the blood clams had experienced extensive flooding in December 1991 and December 1992. In general, there was poor correlation between the width of the semilunar growth increments of the monthly samples and variations in the salinity. This finding is supported by other physiological evidence that *A. granosa* is extremely tolerant to salinity fluctuations (Liong, 1979; Broom, 1982b; Davenport & Wong, 1986) and therefore is capable of living in unstable environments such as estuaries.

Recently Debenay et al (1994) have examined the microgrowth patterns present in thin sections of *A. senilis* living in the upper layers of the sand banks in a Senegal lagoon and attempted to determine the periodicity of the microgrowth bands and to determine the age of samples from their population. In thin section the microgrowth bands appeared as a series of transparent and opaque zones bordered by a thin transparent layer. The

researchers were not be able to ascertain whether the patterns had a tidal or daily periodicity. However, they observed that the growth increments showed a seasonal variation in width. The widest growth increments were deposited during the equinoctial spring tides with an average width of 0.06mm. In this study *A. granosa* showed maximal growth during neap tides (0.18mm.day^{-1}).

Narrowed increments were found in *A. senilis* during the winter (January-February) and rainy season (July-October). Debenay et al (1994) noted that recruitment for this particular species occurred during during the rainy season (see also Okera, 1976). Although these workers did not study the reproductive cycle it is reasonable to suggest that a narrowing of the microgrowth bands during the rainy season may be due to the reproductive activities of the clams similar to that observed in *A. granosa* in this study.

The recorded K values obtained from the literature and shown in Table 22 illustrate that the growth performances are similar amongst populations of *A. granosa* studied from the different regions. The high K values for the populations in Jelutong, Kuala Jarum Mas and Batu Muang, Malaysia, (Pathansali, 1964) presumably occurred because they were calculated from individuals of less than one year old. It is clear that in comparison to other populations, blood clams from Tapak grew very slowly as indicated by the lowest value of K although the maximum asymptotic length was not dissimilar from the population in Wedung. However, the calculated K value for the Wedung populations was almost 2.3 times the value from the Tapak population.

In Malaysia, *A. granosa* was reported to attain 27mm, 37mm and 43mm on completion of 1-3 years growth respectively (Pathansali, 1964). In Kakinada Bay, India, Narasimham (1969) previously stated that this blood clam species attains 31.5mm and 49.5mm after 1 and 2 years of growth. In a more recent study, Narasimham (1988) confirmed that

Table 22: Recorded values for the growth constants (K) and L_∞ from the genus *Anadara* derived from the von Bertalanffy growth equation

Species	Location	K	SE for K	L∞ (mm)	SE for L∞	Authority
A. granosa	* K. Selangor	1.01	0.38	44.40	4.2	Broom, 1982c, 1983c
	•* Jelutong	3.39	1.25	35.90	9.7	Pathansali, 1964
	•* K. Jarum Mas	4.18	0.45	30.30	3.0	Pathansali, 1964
	•* Batu Muang (S)	2.11	0.94	29.60	9.1	Pathansali, 1964
	•* Batu Muang (L)	0.62	0.13	49.60	8.6	Pathansali, 1964
	* Kuala Juru	0.55	n.a.	45.00	n.a.	Ng, 1986
	* P. Sangga Besar	0.87	n.a.	37.40	n.a.	Ng, 1986
	* Kuala Larut	0.79	n.a.	40.50	n.a.	Ng, 1986
	* Kuala Larut	0.60	n.a.	34.20	n.a.	Ng, 1986
	* Sungei Besar	0.78	n.a.	41.40	n.a.	Ng, 1986
	Kakinada Bay India	0.58	n.a.	73.40	n.a.	Narasimham, 1988
	♣ Wedung, Indonesia	0.78	0.55	48.15	0.834	Present study
	♣ Tapak, Indonesia	0.34	0.03	51.75	1.208	Present study
A. senilis	Sierra Leone	0.27	n.a.	99.00	n.a.	Okera, 1976
		0.22	n.a.	145.0	n.a.	Okera, 1976
A. granosa bisenensis	Japan	0.24	0.37	73.3	24.9	Cahn, 1951
A. subcrenata	Japan	0.68	0.83	53.6	9.8	Kusukabe, 1959 cited in Broom, 1985

* Populations of *A. granosa* from different regions in Malaysia,

•* Values derived from published tables in Pathansali (1964). Except for large specimens aged 1-5 years in Batu Muang (Large), the rest were from small specimens aged 7-10 months (Batu Muang Small).

♣ Values generated from measurements of microgrowth increments. The rest of the data were generated from analysis of the modal progression in size frequency distributions.

n.a. data not available, SE Standard Error.

A. granosa in Kakinada Bay grew at even faster rate, i.e. 41.1mm, 55.3mm and 66.3mm following 1 to 3 years of growth with K= 0.58.y^{-1} and L∞= 73.4mm . It would appear from Broom's data (1983c) that specimens of *A. granosa* larger than 53.5mm rarely occurred in the population he studied. Similarly in the present study, large blood clams were rarely encountered with a specimen from Wedung attaining a size of 56.3mm and a single blood clam from Tapak reaching 63mm. In India, Narasimham (1969) regularly found specimens measuring up to 63mm in length, and in 1988 he reported that he had found a clam measuring 71.2mm in his population (Narasimham, 1988). The largest specimen collected by Boonruang and Janekarn (1983) at Phuket, Thailand, was 51mm long, similar to the maximum size recorded for the Wedung, Indonesian, population.

The subspecies *A. granosa bisenensis* apparently exhibits a much slower rate of growth (see abstract in Yoo, 1971), presumably because they occur at higher latitudes than Indonesia, where there are pronounced seasons of growth. Yoo (1971) further reported that in Korea the growing season for *A. granosa bisenensis* extends from June to September with clams reaching a size of 24mm in their first 24 months of growth. The largest specimen he recorded was one of 41.1mm long which is similar to the L∞ value for *A. granosa* in Malaysia (Pathansali, 1964 for the population of large specimens in Batu Muang; Broom, 1983c; Ng, 1986) and the population in Wedung (Table 22). Cahn (1951) studied a population of *A. granosa bisenensis* in Japan and found individuals up to 70mm long, similar to the maximum recorded lengths for *A. granosa* reported by Narasimham (1969, 1988).

A. broughtoni appears to be an extremely rapidly-growing anadarinid unlike *A. granosa bisenensis*. Yoo (1970) showed that grown in hanging culture in Korea *A. broughtoni* grew from 4mm to 48mm in one year. Data on commercially exploited natural populations of these species are however

not available. According to Squires et al (1975), *A. tuberculosa* is a slow-growing but large species. The recorded growth for individuals between 33-64mm was 1mm/month and the largest specimen found measured 110mm. *A. senilis* appeared to grow very slowly (Okera, 1976; Wolff et al, 1987) and may live up to 20 years (Wolff et al, 1987).

To summarise this chapter, the rounded and the elongated forms of *A. granosa* as well as *A. antiquata* show a faster rate of growth laterally than growth in their other linear dimensions in order to achieve a globular shape. It appears that for *A. antiquata* and *A. granosa* in general, the lightest weight shells with the globose shape are probably adapted for living in a muddy habitat. The elongated form of *A. granosa* however, deposited a heavier shell, with finer tubercles and smaller hinge plate than the rounded form which may not aid the animal in stabilising its burrow in the fine sediments. The reason for these is not known and a further study will be necessary to explain these features. *A. antiquata* tends to produce heavier shells than the rounded form of *A. granosa*, and this along with byssus production, are thought to be an adaptation of these blood clams to life in intertidal wave washed areas.

The sampling method used for collecting the monthly samples of blood clams was probably not sensitive enough to collect small spat. Whilst the method of Bhattacharya was able to resolve the population into 4 major cohorts within the *A. antiquata* population from Bandengan, it was not able to identify the recruits. This is unusual, since *A. antiquata* exhibits a more seasonal reproductive cycle than the congener *A. granosa*. Virtually nothing is known about the duration of the pelagic stage of *A. antiquata*, so any back extrapolation for estimating the growth rate of this population can only be approximated. Nonetheless, the analysis showed that *A. antiquata* grows in a relatively slower rate than its congener *A. granosa*.

For *A. granosa*, the method of Bhattacharya confirmed there were 7 distinct cohorts in the population studied and that 3 major recruitments took place between 1991-1993. Using an approximate rate of shell growth obtained from measurements of the microgrowth increments, it was possible to estimate the settlement time of the two most pronounced recruitments first observed in the population in August 1992 and July 1993, as those settling in November/December 1991 and 1992 respectively.

Marked specimens of *A. granosa* grown experimentally in the shallow subtidal in the natural environment, showed a clear daily (= tidal) banding pattern with a neap-spring lunar cycle. Due to the mixed semi diurnal tidal cycle in the area of study it was found that the number of growth bands deposited was equal to the number of days (tides), i.e. one band is deposited every day. The decrease in width of the growth increments in the majority of *A. granosa* shells examined during the peak spawning season in June-August 1993 was found to correlate with a reduction in the gonad index of the blood clams. Although *A. granosa* may have more than one period of spawning within a year accompanied by the continuous release of gametes, it was shown that these spawning periods had an effect on shell growth. Shell growth was reduced and this was characterised by a series of alternating narrow and wide increments in the shell, which were then used to establish marks in the shell and subsequently were used to estimate the age of shells.

The growth constant in the von Bertalanffy equation (K) for the population of *A. granosa* in Wedung is higher than the value in blood clams from Tapak, whilst the maximum asymptotic size ($L\infty$) attained was not much different to the one for the population in Tapak. These values for Wedung are comparable to the values of other population from different localities and latitudes, but the population in Tapak appeared to have the

lowest K values indicating that clams in this particular population are growing slowly.

CHAPTER V

GENERAL DISCUSSION

It is clearly in the interest of all species to reproduce successfully before some environmental uncertainty curtails the reproductive capacity of any generation within the population. Some molluscs begin to breed only after their somatic growth has become increasingly reduced, e.g. the muricid *Ceratostoma foliatum* (Spight et al, 1974) or the bivalves *Macoma balthica* (Gilbert, 1973) and *Modiolus modiolus* (Seed & Brown, 1978), whereas other species continue to grow during and between breeding seasons, for instance the fresh water pulmonate *Lymnaea peregra* (Calow, 1981), the bivalves *Mytilus edulis* (Bayne, 1975, 1976), *Cerastoderma edule* (Seed & Brown, 1978) and the two *Anadara* species studied in this investigation.

A. granosa appears to have evolved a series of life history traits characteristic of a species adapted to a variable environment. It has a high growth rate, matures early and exhibits a higher degree of fecundity compared with its congener *A. antiquata*. The histological study showed that both these *Anadara* species are iteroparous for they may reproduce successfully over several seasons. *A. granosa* can be considered to have a planktotrophic type of development, with females discharging approximately $0.2\text{-}1\text{x}10^6$ small eggs of 55-60μm in diameter even though they appear to have a short period of pelagic life (ca. 1 month). Whilst the population in Wedung has a relatively short life span due to human harvesting when compared to the adjacent population in Tapak, it remains distinctively iteroparous with one spawning peak followed by a low level of spawning over its 1-2 years life span. From the size of the eggs, it is thought that *A.*

antiquata is also an iteroparous species, although the average density of the oocytes appears to be consistently lower than in *A. granosa.* Virtually nothing is known about the early development of this particular species.

Growth in *A. granosa* is maximal in the first year following spatfall within which the majority of clams become sexually mature and spawn, i.e. when they are still relatively small (15mm) compared to their final adult size (63mm). This growth pattern seems to operate through the need to reproduce efficiently within a framework of heavy mortality from two distinct sets of predators, humans and perhaps to a lesser extent natural predators. Moreover, these bivalves thrive in the areas adjacent to mangrove stands in estuaries which are generally considered to be unpredictable habitats.

The drastic effects of predation in Wedung can be inferred from the scarcity of blood clams over 3 years old, although the clams at Tapak can in fact live up to 14 years. As in the case of the common cockle *Cerastoderma edule* in temperate waters (Seed & Brown, 1978), which also experiences constantly heavy predation pressure, escape by growing to a large body size is probably not feasible since all size ranges of these clams and cockles are under intense predation pressure. Thus, the maximum rate of growth during their first year in these bivalves is perhaps intended more towards attaining a minimum size of sexual maturity rather than for escaping predation. In comparison, the K value for the Wedung population is considerably higher (2.3 times) than the population at Tapak, i.e. $0.78.y^{-1}$ compared to $0.34.y^{-1}$, with a slightly smaller maximum asymptotic size (48.15mm and 51.75mm respectively). On the other hand, sexual maturity in *A. antiquata* first occurs at a slightly larger body size than in *A. granosa.* The population of this slower growing species in Bandengan is collected for human consumption only on a subsistence basis. Even so, predation pressure on these clams may

be quite substantial as a result of the relatively high occurrence of brachyuran crabs on the clam beds. There is a possibility, therefore, that the delayed onset of reproductive development in *A. antiquata* has evolved as a strategy to avoid predation. The ability to escape predation by growing too large to be eaten has also been demonstrated in several other species, e.g the opisthobranch *Navanax inermis* (Cooper) (Paine, 1965), the barnacle *Balanus cariosus* (Pallas) (Dayton, 1971), the gastropod *Melampus bidentatus* (Montagu) (Vince et al, 1976) and the mytilids *Mytilus californianus* (Paine, 1976) and *Modiolus modiolus* (Seed & Brown, 1978).

It has been previously recorded that the major spawning season for *A. granosa* varies between years even within adjacent latitudinal localities. In Malaysia clams generally exhibit 1-2 peaks annually with a low intensity of spawning throughout the year, though the second major spawning period frequently does not result in any reported spatfall (Broom, 1983b). Similarly, in Tapak and Wedung *A. granosa* would appear to spawn at a low level all year round but probably with two main spawning periods which results in two annual recruitments. There are however, no records of continuous low levels of spatfall throughout the year either in Malaysia, Wedung or Tapak, which would be consistent with these results. Nevertheless, it has been suggested that selection for such reproductive effort over more than a single annual spawning period may have occurred in certain highly variable environments, where environmental fluctuations greatly influence juvenile survival rates (McMahon, 1983).

By spreading reproduction throughout the year the loss of an entire year's reproductive effort by a bivalve through massive mortality of the new generation as a result of some chance unfavourable environmental change can be prevented. This life history tactic which is known as bet hedging (Stearns,

1976) is observed in *A. granosa* and may help to provide an important conceptual framework for making comparisons with the congener *A. antiquata*. The temporal spreading of reproductive effort may be extremely valuable for the survival of *A. granosa* when set against the catastrophic loss of members of the population. The peak spawning season always coincided with highest salinity and lowest sediment temperature, i.e. during the dry season. However, it appears that the reverse of such environmental factors (lowest salinity and high/highest sediment temperature) during the rainy season also triggers a series of minor spawnings. If spawning in *A. granosa* were restricted only to the dry season, heavy flooding or some other environmental stress could result in the massive loss of juveniles into the deeper subtidal areas due to the outflowing water where they may then be trawled as spat for cultivation further up in the higher tidal zone (in tambaks)

Both flooding displacement and human harvesting, is believed to have been responsible for the removal of clams from the broodstock in the marginally subtidal areas. Therefore, minor spawning periods and low spawning intensity throughout the year, even though the latter did not result in any distinct spatfall, presumably increases the chance that juveniles will survive and reach maturity during periods when environmental conditions are more favourable. This strategy would ensure that a relatively large number of individuals would be available to reproduce in the following year.

It was estimated from back extrapolation (Chapter IV) that the major recruitments of clams in August and March were most likely derived from spat which had settled in April or as early as December the previous year. Histological studies (Chapter III) confirmed that in both December and April there were spawning periods for *A. granosa*. On the other hand, the reproductive cycle in *A. antiquata*, which inhabits more stable environments,

appeared to be more seasonal, since this species may not require a second reproductive period to ensure survival of the breeding stock.

Hermaphroditism and gonochorism are distributed unevenly throughout the major molluscan taxa (Calow, 1983) although most bivalves are gonochoristic (Sastry, 1979). Hermaphroditism is thought to be advantageous in animals with a low mobility and/or those that live in low population densities, because it effectively increases the chances of successful mating contacts (Ghiselin, 1969). However, it also carries a cost because each parent must build and maintain two sets of reproductive material. They will therefore, have fewer resources to invest in gametes and will thus produce fewer eggs and sperm than gonochoristic species (Calow, 1983). *A. granosa* and *A. antiquata* in this study live in relatively low density populations; they are also relatively immobile although they may move closer to each other during the breeding season as indicated by their contagious distribution at that particular time.

Moreover, investigations of gonadal condition of both species in Wedung, Tapak and Bandengan have shown that the smallest sexually mature individuals were males, the majority of older, larger individuals were females, and a few of the intermediate-sized animals were protandric hermaphrodites. It seems likely that being female in general is energetically more costly than being male, and this will be very important in relation to the adaptive value of protandric hermaphroditism, i.e. when the less costly male function precedes the more costly female one (Russell-Hunter & McMahon, 1975).

A general and more meaningful insight into this process in bivalves however, should involve ecological studies with complete sets of data within and between populations on all aspects from their life cycles to population

dynamics, ingestion and assimilation rates, production and reproduction efficiencies as well as genetic composition.

Surface shell sculpturing in gastropods and many bivalves may contribute to traction, protection or shell strengthening. The familiar ribbing, or corrugations of cockle and some scallop shells for example, increases shell strength (Ruppert & Barnes, 1994). Moreover, the presence of strong nodules or knobs on the radial ribs of both valves in *A. granosa* and *A. cuneata* is reported as another adaptive structure for shallow burrowing in soft mud (Lim, 1968). Combined with the absence of any siphons, which require the animal to remain near the surface, the numerous well-developed nodules like those in *A. granosa* serve to increase the area of contact with the surrounding soft mud and subsequently assist in keeping the animal, which is rather large, from sinking too deep into the sediment.

The other species studied, *A. antiquata*, does not have these protruding rib nodules. Instead it has a rather fragile slender byssus stem to attach onto solid objects whilst shallow burrowing in sandy-mud. The sand particles presumably give a better resistance to sinking than soft mud. Allometric study on shells of the two species studied, *A. granosa* and *A. antiquata*, revealed that both tend to grow faster laterally presumably to achieve a more globular shape and somehow maintain a light-weight shell per unit length; both features which are necessary for life in soft sediment.

When comparing the two ecomorphs, i.e. the rounded and the elongated form of *A. granosa* within the Wedung population, the rounded clams were inclined to maintain a negative allometry between their axis of normal height (height 1, see Fig. 28) and length with less emphasis of growth towards the posterior region of the shell (i.e. axis of the maximum dorsal-ventral height or height 1). The result of such growth is the production of a much taller shell

than occurs in the elongated form of comparable length. The axis of normal height and length in the more elongated clams are related isometrically. The axis of maximum height of the elongated form increases at a faster rate than length which may result in a more mytiliform outline. However, that does not appear to occur because width increases at a much faster rate compared to all other linear dimensions. These allometric relationships result in a longer shell of similar height compared to the rounded form. Furthermore, length of the hinge plate increases at a slower rate compared to the maximum dorsal-ventral axis of growth (height 1). The hinge plate dimension however is isometrically related to length and to the normal height axis of the shell and this maintains its elongated and cylindrical date-like shape.

Growth in shell width amongst the rounded clams was also greater than the other linear dimensions of the shell (length, height and hinge axis) resulting in their globular outline in cross section. In both species, *A. granosa* (i.e. rounded form) and *A. antiquata*, shell weight was negatively allometric relative to length, to width, to the axes of normal and maximum height and to the hinge plate. In the elongated form of *A. granosa*, shell weight increased isometrically with respect to length, to the hinge plate and to the axis of normal height but, it was negatively allometric to width and to the axis of maximum shell height.

Even though morphologically the elongated form of *A. granosa* looks similar to the rounded one, allometric studies revealed that the elongated form shares only a few similarities in its shell dimensions to that of the rounded form, than it is to *A. antiquata.* Furthermore, both the rounded and the elongated forms of *A. granosa* exhibit some allometric similarities to *A. antiquata.* The rounded *A. granosa* resembles *A. antiquata* in terms of the linear dimensions of its shell, whereas comparably heavier shell weight and a

less pronounced hinge plate in the elongated form of *A. granosa* are broadly similar to those of *A. antiquata*. Using *A. antiquata* on a basic grid, Lim (1968) attempted to apply the theory of transformations (i.e. for organisms that have different body proportions but are believed to be derived from a similar form) at the generic level of *Anadara*. He concluded that *A. granosa* exhibits a complex shear type of deformation from *A. antiquata* which is the type species for the genus *Anadara* (Oliver, 1992). The findings here thus parallel the taxonomic descriptions given by these authors.

Growth bands in the shell of *A. granosa* have been shown to have a tidal periodicity which reflects the predominantly diurnal component of the mixed semidiurnal tidal regime in the area. These growth bands show a pattern which has a semilunar periodicity in which narrower growth increments are produced during or a few day after spring tides whilst maximum growth occurs during neap tides. These findings are consistent with those previously documented for *A. granosa* in Penang, Malaysia (Richardson, 1987). The width of the growth increments was also shown to vary seasonally with narrow (slow growth) increments deposited during February-March and August-September whilst the widest (maximal growth) were formed during May-June.

From a comparison with environmental factors and the reproductive condition of the blood clams, it was found that the deposition of narrower increments was related to the reproductive activity of the clams and not directly to such factors as salinity or temperature. The pattern of narrow and wide increments was used to estimate the age of individual clams and the age structure of the overall population. The results showed that the population at Wedung mainly consisted of young individuals of 1-4 years old and

confirmed the findings from the size frequency distributions, whilst the majority of the population at Tapak contained 5-12 years old clams.

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APPENDICES

Appendix 1

Reagents for Oxygen analyses

1. Manganous sulphate solution: 450 g of $MnSO_4.4H_2O$ are dissolved in distilled water, diluted to 1 l and stored in a glass stoppered bottle.

2. Alkaline iodide solution (Sodium hydroxide 8N/Iodide 3M): 320 g of NaOH are added to about 400 ml of distilled water in a 2 l conical flask. It was mixed vigorously by swirling. The solution was made up to 1 l and stored in a glass bottle which must be stoppered with a plastic or rubber stopper, but not a glass stopper.

3. Sulphuric acid (10N) solution: 280 ml of analytical grade concentrated sulphuric acid was carefully added to 500 ml distilled water, cooled and made up to 1 l.

4. Starch solution: 5 g of soluble starch was mixed in 10 ml cold distilled water and poured into 200 ml boiling water.

5. 0.005 M Sodium thiosulphate solution: about 1.24 g of $Na_2S_2O_3.5H_2O$ were dissolved in 1 l distilled water. If the pH of the distilled water is low because of the dissolved carbon dioxide, a colloidal haze of sulphur will result. To overcome this, one pellet of sodium hydroxide was added.

6. 0.001667M Potassium iodate standard solution: About 0.5 g of analytical grade KIO_3 was oven dried from 1 - 2 h in oven at 110°C. An exact amount i.e.: 0.3567 g or as close to 0.3567 g (W g) as is reasonably possible was weighed and transferred to a beaker to be dissolved up to exactly 1000 ml with distilled water. A solution containing 0.3567 g/l has a molarity of 0.001667. Thus the molarity of the iodate standard is:

$$0.001667 \times W/0.3567$$

Standardisation of thiosulphate for oxygen analyses

The Winkler procedure uses thiosulphate as titrant. Thiosulphate itself cannot be obtained or prepared with very high purity and cannot therefore be used as a primary standard. Accordingly, it must first be standardized with a solution whose concentration can be calculated accurately. A standard solution of potassium iodate was used for this purpose.

To about 50 ml of seawater in a conical flask was added 1 ml of each of sulphuric acid and alkaline iodide (reagents no2 and no3) respectively and solution thoroughly mixed. At this stage the solution should not contain iodine, which can be checked by adding starch to a preliminary sample. Following the additions, 10 ml of the potassium iodate standard (reagent no. 6) was added to the solution using a volumetric pipette. The solution was then covered and allowed to stand for 10 min to accomplish the reaction. Finally, it was titrated with thiosulphate. The molarity of thiosulphate (M) was calculated as follows:

$$M = (6 \times V_2 \times M_1)/V_1$$

where: V_2 is volume of iodate added (ml)

M_1 is molarity of iodate standard

V_1 is volume of the thiosulphate added (ml)

Determination of BOD bottle's volume

The exact volume of 100 ml oxygen bottles was determined by weighing them empty and full of water using a fine Mettler balance. The volume of the bottle was calculated by difference, assuming a water density of 1.0. The figures was then etched on the bottle. This should remain correct provided that the bottles are always used with the same top.

Appendix 2

Apparatus and reagent for particle size analyses (Piper, 1950)

1. Sieve: 70 mesh size (≡180 μm) standard stainless-steel sieve.
2. Sedimentation cylinders: Pyrex cylinders of 40 cm height and approximately 6.5 cm internal diameter. The cylinders are fitted by thick rubber bungs to ensure a watertight joint.
3. Pipettes: with specially lengthened lower stems. Specifications of a pipette suitable for such purpose are as follows:
   - lower stem: to be 39 cm in length and to have a ring etched around it at a distance of 280 mm from the lower tip,
   - upper stem: to have the usual dimensions,
   - time of delivery: to be within 25 - 30 seconds and to be marked on each pipette.

   Each pipette was marked to correspond to a given cylinder. A 1 cm length of thick-walled rubber tubing fitted tightly on the lower stem and it was adjusted so that when the lower stem is passed through the cork block (below) placed on the sedimentation cylinder, the etched mark will be on the surface of the suspension; indicating that the tip of the pipette is the correct distance below the surface.

   For the second pipette sample, the surface of the suspension will be lower than the graduation mark on the cylinder, owing to the withdrawal of 25 ml of suspension for the first sample. Allowance should be made for this when adjusting the pipette for the second sample.
4. Cork block: A piece of 2 cm thick compressed cork sheet was cut to a 10 cm circle and a hole was drilled through the centre, 6 mm in diameter or just large enough to take the lower stem of the pipettes freely. This piece of equipment facilitating stable position of pipette during suctions.
5. Reagent: 10% weight of Sodium polyphosphate, 10 g of $(NaPO_3)_6$ was added to 90 g of distilled water in a graduated cylinder and stirred thoroughly until all the solute dissolved. It usually making up to only 95 ml in volume, so that 10 ml of this solution contained: 10ml/95 ml x 10 g = 1.0526 g $(NaPO_3)_6$.
6. Evaporating dish: made from thin aluminium sheet, 64 mm in diameter and 19 mm deep, fitted with a cover.

Table 1 App.2: The time of sedimentation at different temperature. International System (Piper, 1950)

Temperature °C	Fine Sand Decantation Depth 28cm		First Pipette Sample Depth 28cm		Second Pipette Sample Depth 22cm		Depth 28cm	
	Mins	Secs	Mins	Secs	Hours	Mins	Hours	Mins
10	6	20	17	30	23	0	-	-
11	6	10	17	0	22	15	-	-
12	6	0	16	30	21	45	-	-
13	5	50	16	15	21	15	-	-
14	5	40	15	45	20	30	-	-
15	5	30	15	15	20	0	-	-
16	5	20	15	0	19	30	24	45
17	5	10	14	30	19	0	24	15
18	5	0	14	15	18	30	23	30
19	5	0	13	45	18	0	23	0
20	4	48	13	30	17	30	22	30
21	4	40	13	15	17	15	22	0
22	4	30	13	0	16	45	21	30
23	4	30	12	30	-	-	21	0
24	4	20	12	15	-	-	20	30
25	4	15	12	0	-	-	20	0
26	4	10	11	45	-	-	19	30
27	4	5	11	30	-	-	19	0
28	4	0	11	15	-	-	18	30
29	3	55	11	0	-	-	18	15
30	3	50	10	45	-	-	17	45
31	3	45	10	30	-	-	17	30
32	3	40	10	15	-	-	17	0
33	3	35	10	0	-	-	16	45

Appendix 3

Bhattacharya's method

Probit analysis forms the basis of this method which proceeds to resolve Gaussian distribution mixtures as follows:

Size frequency data are transformed into logarithmic differences of adjacent class frequencies and plotted against the midpoint of each size class. Points constituting a particular component distribution will be aligned along a linear segment. Different age classes will be viewed as adjacent lines of negative slope separated along a size gradient (the x-axis). Having displayed the plotted points, the programme prompts the user to highlight elements to be included in the first component segment, i.e. the line with highest r^2 and SI (separation index) values. The separation index quantifies the extent of overlap between adjacent classes and gives the user a warning when the lower critical value of two for meaningful separation is surpassed.

A regression is then performed, the calculated line is fitted to selected points; and mean, standard deviation, component size (i.e.: the number of individuals contained) and r^2 statistics are displayed. The derived normal distribution is superimposed on the actual size frequency histogram for visual comparison. Mean size and standard deviation parameters of the age classes are derived respectively from the x-intercept and slope of its regression. The number of individuals contained in this group is back-calculated from initial size-frequency data given the new established class boundaries.

The user is then given the option of repeating the analysis for newly selected points along the same segment, if unsatisfied with a poorly fitting regression, or because of the exclusion of too many data points, or an unsuitable fit of the superimposed distribution. This entire procedure is repeated for all linear segments. However, not all plotted points will lie discretely on such linear segments, this being partly due to the degree of component distribution overlap. Outliers may be visualized as the tail ends of overlapping adjacent distributions. Although the criteria for outlier omissions are somewhat arbitrary, the interactive nature of the point selection procedure combined with the subsequent statistical testing by ELEFAN reduces subjectivity to a minimum. A composite distribution is then calculated from the sum of all overlapping components' frequencies over the entire size spectrum plotted against size classes superimposed on the initial histogram, and a χ^2-test between these distributions is performed.

Appendix 4

Periodogram Analysis

The periodogram analysis was run in the Perio Software (written by Alf Aagaard, Ecotoxicology, Institute of Biology, University of Odense, Denmark) according to Warman (1990). Significance of a periodicity is given by randomising the original data set. The periodogram of the randomised data approximates a straight line as all inherent periodicity is lost. The randomised raw data set were then used to calculate a number of mean values, $x_{(p,t)}$, for data sets all of which contain data points separated by a certain period (p) but where the individual data sets start at a different time (t). When all possible values of $x_{(p,t)}$ have been calculated, these individual means were then considered as individual values from which an overall mean $X_{(p)}$ thus calculated. The standard deviation of this overall mean was then plotted for the periodogram at period = p. The individual mean value is low if all data points were individually low, and high if individual data points used are large. Subsequently, the overall standard deviation will be large as a function of the wide spread of data. High values of the periodogram statistics occur when the period under test approximates to the periodicity inherent in the raw data. By calculating the least squares regression line for all the periodogram statistics within the range, a straight line with upper and lower 95% confidence limit is produced. Significant periodicity is assumed when the periodogram statistics for a given period is greater than the upper 95% confidence limit of the randomised data regression line at that point.