

Interrelations between hydrographic conditions, nanoplankton and bivalve larvae in the Mali Ston bay (Southern Adriatic)

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The Mali Ston bay (the southern Adriatic) is a promising shellfish farming region, where a good survival of bivalve larvae is possible due to relatively stable hydrographic and favourable feeding conditions. If compared with other locations along the eastern Adriatic coast, the Mali Ston bay is relatively rich in nanoplankton cells, predominantly 2 - 5.7 μm in diameter, as the most appropriate food for bivalve larvae. Detritus particles of up to 10 μm in size coated with adsorbable organic matter and attached bacteria are supposed to be important source of food for bivalve larvae in spring.

INTRODUCTION

Ecological studies of the bivalve larvae along the eastern Adriatic coast have begun in the last two decades and have been mostly concentrated in two areas: the Lim Channel (HRS-BRENKO, 1971, 1973, 1977, 1980) and Mali Ston bay (MOROVIĆ and ŠIMUNOVIĆ, 1980; STJEPČEVIĆ *et al.*, 1981; MUŠIN, 1986). In the Mali Ston bay, where mussels and European oysters have been traditionally cultivated, scientific attempts at describing the relationship between bivalve larvae populations and other ecological factors have still not been made. The distribution of bivalve larvae was not followed continuously all the year round, but occasional investigations only included the relations between bivalve larvae and thermohaline properties of the seawater in the inner part of the Mali Ston bay (MOROVIĆ and ŠIMUNOVIĆ, 1980; STJEPČEVIĆ *et al.*, 1981). Numerous plankton samples were collected

during monthly investigations through 1979/1980, carried out by the staff of the Biological Institute, Dubrovnik. Temperature, salinity and currents were also measured (BALENOVIĆ, 1981). The aim of this paper is to analyze temporal and spatial distribution patterns of the ecological characteristics along with the distribution of the bivalve larvae in the Mali Ston bay, through 1979/1980. Results presented in this paper, including subsequently collected data are the first steps in making out an ecological model which may be used by the shellfishermen to successfully estimate the possible production of oysters and mussels in this area.

Study area

The Mali Ston bay is a scarcely inhabited, closed, well-indented area, with shores covered with dense Eumediterranean vegetation. The continental area closer to the coast is composed

of a high permeable limestone mass having specific hydrogeological characteristics (BAHUN, 1981). The groundwater drainage towards the sea and the underground connections between ponors (swallow holes) and vruljas (submarine springs) is intensive especially during the rainy periods. The Mali Ston bay extends towards NW where there is a considerable freshwater influx from the Neretva river.

According to its hydrographic conditions and phytoplankton photosynthetic rate, the Mali Ston bay belongs to highly productive Adriatic coastal ecosystems (BULJAN, 1964; PUCHER-PETKOVIĆ and ZORE-ARMANDA, 1973), whereas on the basis of the phytoplankton biomass it is included into moderately eutrophicated ecosystem category (VILIČIĆ, 1989).

Because of the expansion of mariculture and increasing inflow of sewage waters that has resulted from the intensive building up of tourist facilities, the common sewage system that takes all the wastes from adjacent settlements to the open sea was constructed in 1988, protecting the ecosystem from the antropogenous eutrophication.

MATERIALS AND METHODS

Samples for plankton analysis and salinity determinations were taken at five stations along the Mali Ston bay (Fig. 1). Samples were collected once a month throughout the year, from July 1979 to July 1980. Samples for quantitative phytoplankton analysis, bacterioplankton and salinity were taken with Nansen reversing bottles at the depths of 0.5, 5, 10, 20 and 40 m (depending upon the depth of the water column sampled). Phytoplankton samples were preserved in a two percent neutralized formaldehyde solution. The cell counts were obtained by the inverted microscope method (ÜTERMÖHL, 1958). Samples of 25 ml were analyzed microscopically after a sedimentation time of 24 hours. Nanoplankton cells (2 - 20 μm in size) were counted in 20 - 30 randomly selected fields of vision along the counting chamber base plate, under the magnification of 320 X. To avoid miscountings, only easily identifiable nanoplankton cells were counted. Precision of the counting method was about ± 10 percent. However, when

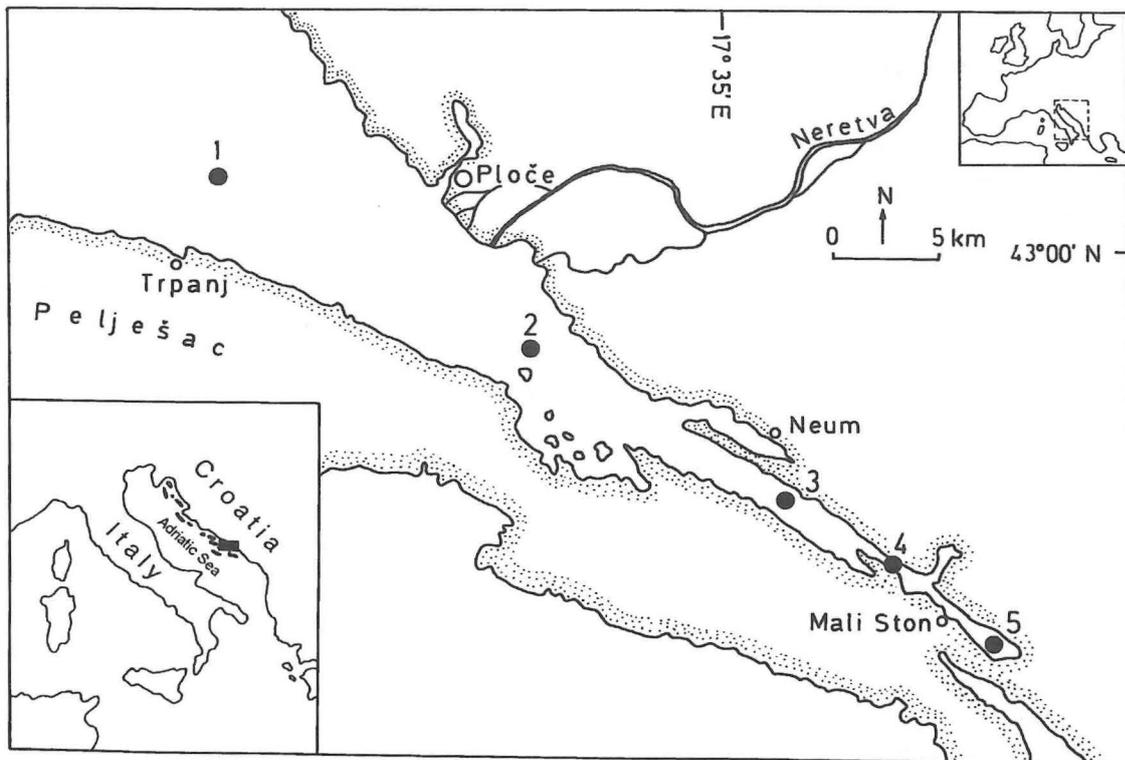


Fig. 1. A map of the Mali Ston bay region and position of stations

detrital particles were present in large numbers, it reached ± 20 percent for nanoplankton. Phytoplankton biomass was expressed as a volume of cells in μm^3 per liter, which corresponds to fresh weight in μg per liter (SMAYDA, 1978). More detailed information on the determination of volume-biomass has been published earlier (VILIČIĆ, 1985).

The counting of heterotrophic bacteria was performed from the surface samples, on agar plates. The growth media contained the sea water (1000 ml), peptone (5 g), yeast extract (1 g), Fe PO_4 (0.1 g) and agar (15 g). The colonies were counted after 20 days of incubation at 22°C .

Samples for the determination of the bivalve population density were collected by a 5 l Van Dorn sampler and fixed in a 2.5 percent formaldehyde solution. After sedimentation and decanting, samples volume was reduced and microscopic counts made under the magnification of 100 X (KRŠINIĆ, 1980). In taxonomic work, the larval shape and size has been examined by LOSANOFF *et al.* (1966), and HRS-BRENKO (1969, 1977).

Currents were measured by Eckmann flowmeter. At Station 4 (Usko) eight measurements were performed daily, except in May 1980 when performed only once for 24 hours. Measurements at each of the depths lasted for 10 minutes.

Salinity was determined by the argentometric titration method standardized against Copenhagen standard sea water (KNUDSEN, 1901; OXNER, 1920). Temperatures were measured with reversing thermometers, and transparency with a Secchi disc of 30 cm diameter.

RESULTS

The variations of different ecological parameters, and the quantity of the bivalve larvae at five Mali Ston bay stations, throughout 1979/1980 are presented in Fig. 2. Increased rainfall (150 - 200 mm monthly) was registered during the period from November 1979 to May 1980 (except in February). This resulted in a

considerably decreased surface salinity in the inner part of the bay in the period from March to May. Temperatures were low and ranged from 10.3 to 13.2°C . A slight increase in temperature in the surface layer began in March (12.4 to 13.7°C).

Two (spring and autumn) phytoplankton biomass maxima were registered. In 1980, biomass started to increase in March. In general, microplankton and nanoplankton biomass as well as bacterioplankton population density curves run approximately parallel throughout the year.

Maximum population density of bivalve larvae were recorded in the late March (81200 ind. m^{-3}), during relatively low seawater temperatures (12.6 to 13.7°C). As many as 94 percent of larvae ($< 180 \mu\text{m}$) presumably belonged to *Mytilidae* (predominately *Mytilus*). The 6 percent of the larvae longer than $180 \mu\text{m}$ presumably contained oyster larvae. In the late June, sea water temperature in the Mali Ston bay reached 20°C , and in the July and August, an increased contribution (26 - 50 percent) of the larvae larger than $180 \mu\text{m}$ was registered that might be due to the increased spawning activity of oysters. The pronounced mussel spawning in March 1980 was preceded by increased rainfall.

The increased population density of bivalve larvae was registered at Station 4. In March, May and July it exceeded 10^4 ind. m^{-3} . In this period detailed ecological analysis was carried out (Figs. 3, 4 and 5). The March decline in salinity arose from the activity of underwater springs in the inner part of the bay. In May and July a considerable freshwater influx came from the Neretva River, with the lowest salinity values being registered at Station 2. Moderate west wind was recorded during the March, May and July field works. The salt water near the bottom ($S > 38 \times 10^{-3}$) was expelled by the outgoing current. In the inner part of the bay (at Stations 3, 4 and 5) the increased nanoplankton quantities were observed ($8 - 34 \times 10^7 \mu\text{m}^3 \text{ l}^{-1}$, $2 - 5 \times 10^6$ cells l^{-1} in March and May, and $1.7 - 13 \times 10^7 \mu\text{m}^3 \text{ l}^{-1}$, $4 \times 10^5 - 1.5 \times 10^6$ cells l^{-1} in July).

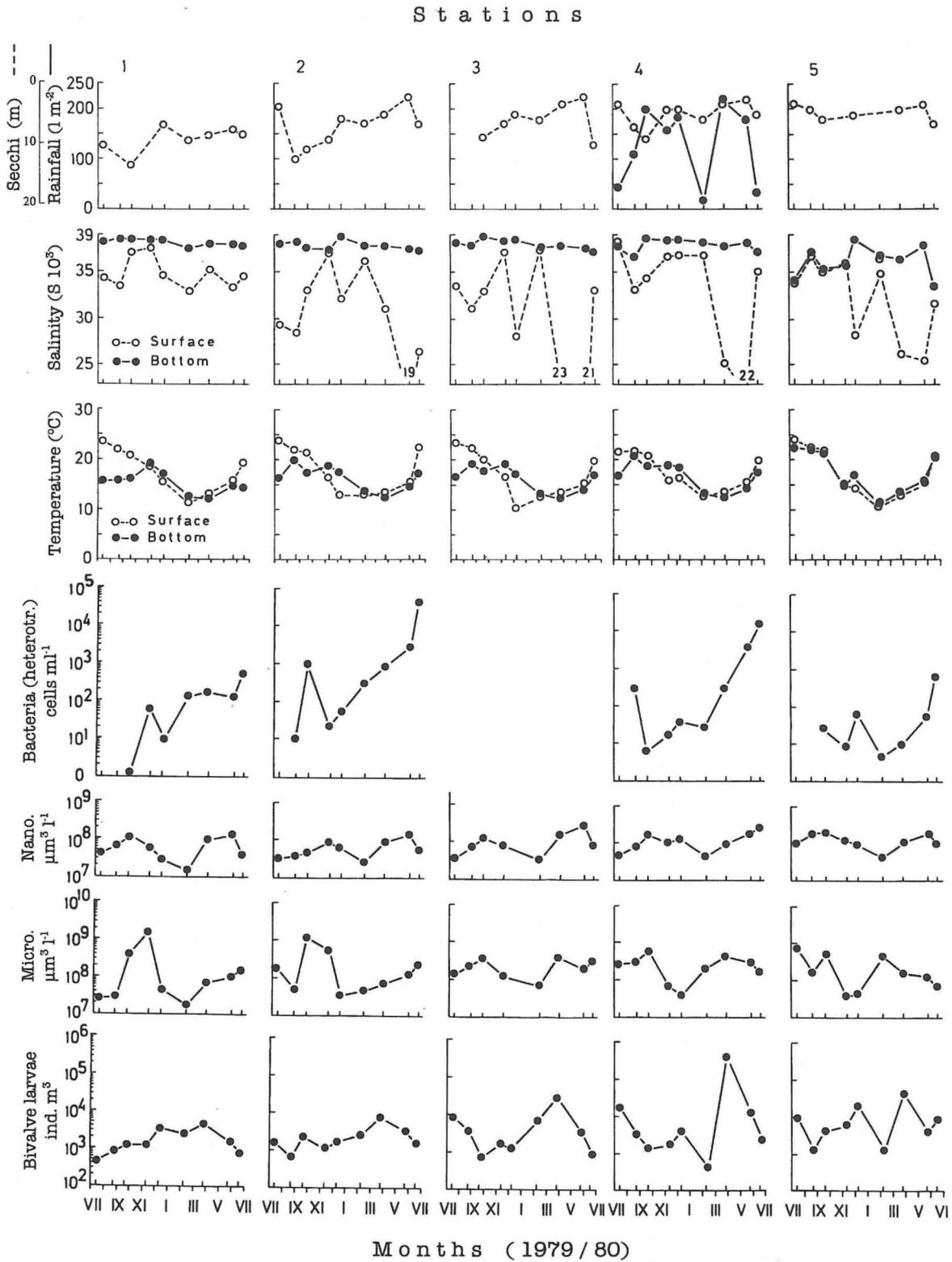


Fig. 2. Variations of abiotic (rainfall, transparency, salinity, temperature) and biotic ecological factors (population density of heterotrophic bacteria, nano- and microphytoplankton biomass, population density of bivalve larvae) at five investigated stations in the Mali Ston bay, in the period from July 1979 to July 1980. Mean values of plankton quantity for the water column are presented.

In March 1980, due to a considerable decrease in the surface salinity at Stations 3 and 4, the pelagic community destabilized and nanoplankton biomass decreased to less than $15 \times 10^7 \mu\text{m}^3 \text{l}^{-1}$ (Fig. 3). The incoming current ($3 - 20 \text{ cm sec}^{-1}$) prevailed at the surface due to a moderate west wind. At the 5 m depth the outgoing current ($3 - 35 \text{ cm sec}^{-1}$) was evident. Nanoplankton was more abundant in the deeper layer (below the depth of 5 m) within the salinity range from 37 to 38×10^{-3} . In the layer below 10 m the currents were weaker and of inconstant direction, thus slowing down the exchange of water masses at this depth. Since the euphotic layer reaches approximately 12 m depth (compare Fig. 2), the photosynthetic activity of nanoplankton is possible throughout the water

column. Low salinity was responsible for a reduced number of bivalve larvae at the surface. On the other hand an increase in their numbers in the deeper, more saline layers was recorded.

In May 1980 and July 1979, the accumulation of nanoplankton populations and bivalve larvae was recorded in the middle layer (approximately 5 m depth), within the salinity range from 36 to 37.8×10^{-3} (Figs. 4 and 5). Weak and inconstant currents in May, and more pronounced surface incoming currents (in the 0 - 5 m layer), and the outgoing near-the-bottom currents in July, were recorded. Nanoplankton biomass in the inner part of the Mali Ston bay was $20 - 30 \times 10^7 \mu\text{m}^3 \text{l}^{-1}$ in May, and $3 - 10 \times 10^7 \mu\text{m}^3 \text{l}^{-1}$ in July. The population density of bivalve larvae was higher in July ($5 - 40 \times 10^3$) than in May ($2 - 10 \times 10^3 \text{ ind. m}^{-3}$).

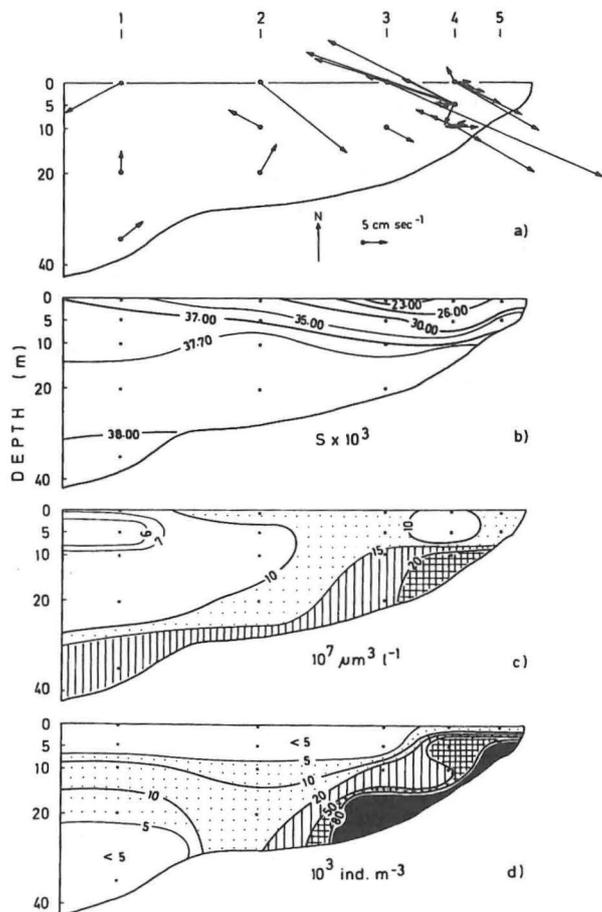


Fig. 3. Distribution of currents (a), salinity (b), nanoplankton biomass (c) and population density of bivalve larvae (d) along the Mali Ston bay profile in March 1980.

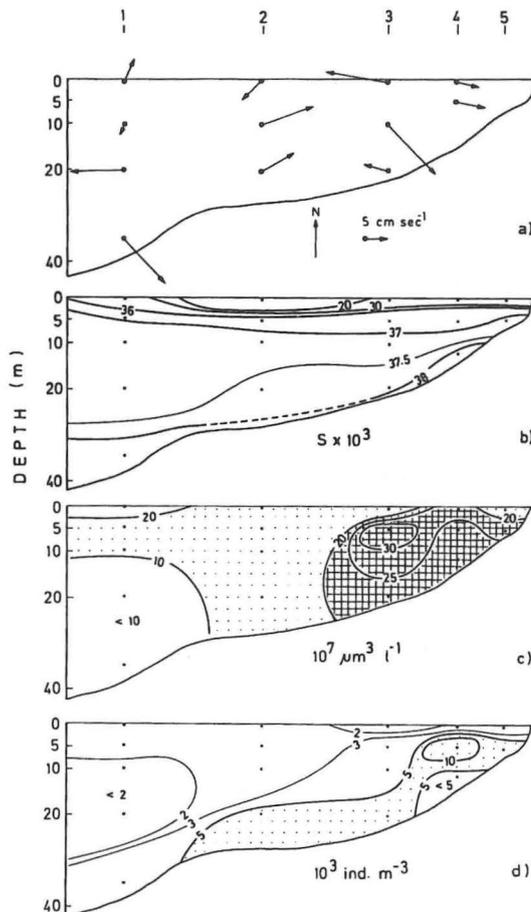


Fig. 4. Distribution of currents (a), salinity (b), nanoplankton biomass (c) and population density of bivalve larvae (d) along the Mali Ston bay profile in May 1980.

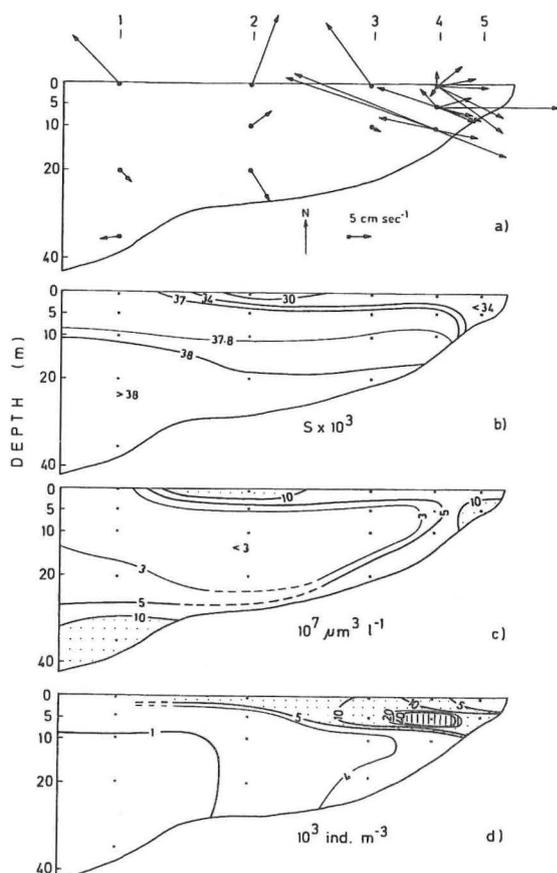


Fig. 5. Distribution of currents (a), salinity (b), nanoplankton biomass (c) and population density of bivalve larvae (d) along the Mali Ston bay profile in July 1979.

DISCUSSION

The inner part of the Mali Ston bay abounds in natural beds of mussels (*Mytilus galloprovincialis* Lmk.) and oysters (*Ostrea edulis* L.), where they have been intensively cultivated. Their larvae dominate the other shellfish, and may be found in plankton throughout a year. Noah's arcs (*Arca noae* L.) and date shells (*Lithophaga lithophaga* L.) are present in lower numbers. The mussels spawn intensively in the Mali Ston bay from February to June (BOLOTIN, 1988). Most intensive spawning activity of the oysters was registered in May, August and September (MOROVIĆ and ŠIMUNOVIĆ, 1980).

Due to relatively dense mussel beds on one hand, and good survival of larvae on the other, the larval populations recorded in the

Mali Ston bay in March 1980 were 100 times those recorded in the shellfish rearing sites in the northern Adriatic. The contribution of the bivalve larvae to the total Mali Ston bay microzooplankton exceeds at times 50 percent (RUDENJAK - LUKENDA, 1985).

Amongst factors affecting larval growth, temperature, salinity and nutrition have received most attention. Survival of *Mytilus edulis* larvae was found as being good throughout a rather wide range of salinities and temperature, with the optimum growth at 20 °C in salinities from 20 to 35 x 10⁻³ (HRS - BRENKO and CALABRESE, 1969). Similar result was obtained for *Mytilus galloprovincialis* larvae which exhibited best growth at 20 °C and 35 x 10⁻³ S (HISS *et al.*, 1989). In the northern Adriatic, successful development and survival of mussel larvae have occurred at salinities 30 - 35 x 10⁻³ and temperatures > 10 °C (HRS - BRENKO, 1977).

BAYNE (1965) recorded a general increase in the rate of growth (shell length) with an increase in temperature. Laboratory experiments have indicated that oysters required higher salinities for larval development to settling size than mussels (> 22 x 10⁻³). Oyster larvae have been found in the Adriatic plankton early in June as a result of sea water temperature exceeding 20 °C (HRS - BRENKO, 1980). June temperature values in the Mali Ston bay also reached 20 °C, which may result in a more intense spawning activity of oysters. Greatest accumulations of the bivalve larvae in the Mali Ston bay usually occurred in the 36 to 38 x 10⁻³, in March in the 27.0 to 37.8 x 10⁻³ salinity range (Table 1). Favorable thermohaline condi-

Table 1. Range of temperature, salinity and phytoplankton quantity in the layers of maximum abundance of bivalve larvae, at three inner Mali Ston Bay stations (3, 4, 5), in March 1980.

Temperature (°C)	12.5 - 13.5
Salinity (S x 10 ³)	27.03 - 37.84
Nanoplankton	
(cells l ⁻¹)	1.8 x 10 ⁶ - 5.0 x 10 ⁶
(μm ³ l ⁻¹)	8.2 x 10 ⁷ - 2.2 x 10 ⁸
Phytoplankton-total (μm ³ l ⁻¹)	2.6 x 10 ⁸ - 1.1 x 10 ⁹
Bivalve larvae (ind. m ⁻³)	8.0 x 10 ³ - 9.9 x 10 ⁴

tions for larval development persist throughout the year, mainly at the approximate depth of 5 m. However, the accumulation of larvae in deeper layers of the water column may result from a change from positive to negative phototaxis in the pediveliger stage (BAYNE, 1964).

Nutrition has the greatest effect on the larval development (HISS *et al.*, 1989). WALNE (1965) demonstrated that nanoplankton cells 3 to 10 μm in diameter were efficiently caught by *Ostrea edulis* larvae. The bivalve larvae presumably feed on microzooplankton as well (< 10 μm in size), but in the Mali Ston bay, this fraction has not been sampled until now. WILSON (1979) found feeding efficiencies of *Ostrea edulis* larvae to be low for particles smaller than 3 μm . An upper limit of retention of approximately 10 μm is imposed by the size of the larval oesophagus (THORSON, 1950). Larvae fed at higher concentrations of *Isochrysis galbana* reach the pediveliger stage sooner than those at the lower concentrations (BAYNE, 1965). DAVIES and GUILLARD (1958) found that a mixture of flagellates supported faster growth of bivalve larvae than either *Monochrysis* or *Isochrysis* individually. They compared the duration of the larval life of mussels in the field and in the laboratory at similar temperatures and indicated a shorter duration in the field. These differences might be explained by the variety of foods available to the larvae under natural conditions. The data resulting from the phytoplankton sampling at the 14 stations along the eastern Adriatic coast (VILIČIĆ, 1989), indicated that the Mali Ston bay is an area relatively rich in nanoplankton. The surrounding land is covered by a dense vegetation, and the terrigenous input of organic matter into the bay probably stimulates the development of nanoplankton population and favors feeding of bivalve larvae. According to WILSON (1980), maximum filtration rate of *Ostrea edulis* larvae at 26 °C occurs at concentration of approximately $3.8 \times 10^9 \mu\text{m}^3 \text{ l}^{-1}$ (cca. 10^8 cells l^{-1}) of *Isochrysis galbana* (4.2 μm modal cell size), and $4.2 \times 10^9 \mu\text{m}^3 \text{ l}^{-1}$ (cca. 5.6×10^7 cells l^{-1}) of *Dunaliella tertiolecta* (5.3 μm modal cell size). Grazing activity of moluscan larvae decreases below 10^7 nanoplankton cells l^{-1} (GALLAGER and MANN, 1980), but at lower cell

densities than optimum, larvae select the larger cell sizes (WILSON, *op. cit.*). Nanoplankton biomass and cell densities in the Mali Ston bay ranging from 8×10^7 to $3.4 \times 10^8 \mu\text{m}^3 \text{ l}^{-1}$ ($2 - 5 \times 10^6$ cells l^{-1}) might be probably underestimated due to the cells being preserved with formaldehyde and not lugol solution. Considering results obtained by HEWES *et al.* (1984), in formaldehyde preserved samples nearly 30 percent of naked flagellates and monads 2 - 4 μm in size are lost, but no loss is recorded in the > 5 μm size fraction. If any of estimated nanoplankton cell density value is increased by 30 percent, optimum food concentration determined in the WILSON's experiment is not achieved, but the quantity calculated may be regarded as more than adequate to sustain larval growth in the Mali Ston bay, where 90 percent of nanoplankton cells belong to the 2 - 5.7 μm , whereas 30 percent of the cells to the 3.9 - 5.7 μm size fraction (VILIČIĆ, 1985). Higher nanoplankton, microphytoplankton and microzooplankton biomass occurred most frequently at stations 3 and 4 (VILIČIĆ, 1981; KRŠINIĆ and MUŠIN, 1981). In the same region high quantities of bivalve larvae were registered, indicating their good survival.

A moderate increase in large- and small-sized phytoplankton biomass, as well as in a number of heterotrophic bacteria was observed in March 1980. Increased accumulations of nonliving particles (determined by measuring particle's number and surface according to LENZ, 1968) most frequently occurred at station 4, in the 5 - 8 m layer, where salinity gradient was most pronounced, as in estuaries (VILIČIĆ *et al.*, 1989). Although the most frequent size of bacteria was < 3 μm , there are some indications that bivalve larvae feed on bacteria (MASSON, 1977). Bivalve larvae feed also on detritus particles < 10 μm in size and bacteria that are attached to them (LANGDON and NEWELL, 1990). They indicated that utilization of bacteria as a food source could make a significant contribution to the nitrogen and carbon requirements of oysters and mussels. Unfortunately, no data are available on the quantity of the free and attached bacteria for the 1979/1980. Bacteria

attached to particles < 10 µm in size were observed in large quantities in the Mali Ston bay by epifluorescent microscopy in 1988 (RADONIČIĆ, 1992). Free bacteria usually dominate over attached ones, except in some estuaries (GOULDER, 1977). The increase in bacterial number in spring 1980 might be related to phytoplankton degradation. In this period, the release of dissolved organic matter during the degradation of phytoplankton may have influenced larval survival through the feeding mechanism (for references see: MANN, 1988). A significant fraction of the dissolved organic matter is converted to particulate organic matter by physical - chemical processes that occur in the layers with an increased salinity gradient (ŽUTIĆ and TOMAIĆ, 1988). Moreover, inorganic particles are almost immediately coated with organic film in waters enriched with dissolved organic matter (ŽUTIĆ *et al.*, 1984). Bivalve larvae probably directly consume and feed on smaller organically-enriched inorganic and detrital particles.

Growth of bivalve larvae may be inhibited by a growth - inhibiting substances produced by phytoplankton in a period of intensive bloom (LOSANOFF, 1954). However, the Mali Ston bay belongs to moderately eutrophicated and ecologically stable ecosystems where excessive phytoplankton blooms are not likely to occur (VILIČIĆ, 1989), making it potentially important shellfish rearing site.

ACKNOWLEDGEMENTS

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Odnos između hidrografskih uvjeta, nanoplanktona i ličinki školjkaša u Malostonskom zaljevu (južni Jadran)

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KRATKI SADRŽAJ

Malostonski zaljev je umjereno eutrofizirano i ekološki stabilno područje u kojem je količina nanoplanktona relativno velika. Krivulje biomase mikroplanktona i nanoplanktona uglavnom se kreću paralelno, a u glavnim crtama ih prati i gustoća populacija heterotrofnih bakterija. Maksimalni broj ličinki školjkaša iz skupine *Mytilidae* zabilježen je krajem ožujka 1980, u vrijeme relativno niskih temperatura mora (12.6 - 13.7 °C). Krajem lipnja, kada temperatura mora u Malostonskom zaljevu dostiže 20 °C, te u srpnju i kolovozu, zabilježen je povećan udio ličinki kamenica. Prostorna raspodjela ličinki školjkaša i njihovo akumuliranje u određenom sloju ovisi o njihovoj starosti (težini), temperaturi, izvoru hrane (suspendirane organske čestice i otopljena organska tvar) i salinitetu (položaju graničnog sloja koji razdvaja površinsku zaslađenu vodu i dublju slaniju vodenu masu).