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Some effects of salinity on survival of early developmental stages of gilthead sea bream (*Sparus aurata* L.)

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Starving gilthead sea bream yolk-sac larvae showed better survival on low (5-25 ppt) than ambient (38 ppt) salinity. Best survival of rotifer fed larvae, from hatching to day 22, occurred at reduced salinity from 38 ppt (ambient) to 15-30 ppt.

INTRODUCTION

Gilthead sea bream (*Sparus aurata* L.) is a euryhaline and eurythermal, carnivorous fish of high market value in the Mediterranean. Apart from European sea bass (*Dicentrarchus labrax* L.) these are the fish most frequently cultured in the Mediterranean. They belong to the family Sparidae, which is found throughout Mediterranean. In the Atlantic Ocean their range extends from the British coast to the Canary Islands. They rarely occur in the Black Sea (BAUCHOT and HUREAU, 1986). Gilthead sea bream are mainly caught in lagoons (of Italy, France, Spain, Tunisia, Israel and some others), coastal waters and river estuaries. Young and adult fish are attracted to brackish waters and hypersaline lagoons of the Mediterranean Sea (BEN TUVIA, 1979).

The first reports on young gilthead sea bream preference for brackish water, estuaries and lagoons can be traced back to RAFFAELE (1888) and was later confirmed by the studies of LO BIANCO (1909) and RANZI (1930). HOLLIDAY (1969) reported salinity to affect general activity, survival and distribution of a large number of fishes. KINNE (1960) demonstrated the effects of salinity on food intake, food conversion and growth. However, salinity is a single factor affecting survival of early life stages of marine fish (ROSENTHAL and ALDERDICE, 1976).

Adaptability of juvenile gilthead sea bream to various temperatures and salinities was studied by AUDOUIN (1962) who attempted to establish maximum and minimum values at which the species could survive. CHERVINSKI (1979) reported that young gilthead sea bream were easily adapted to very low salinities and confirmed this capacity also for later larval stages (CHERVINSKI, 1984). Studies of salinities for optimum survival of eggs and larvae at hatching and yolk sac absorption for gilthead sea bream were also carried out by FREDDI *et al.* (1981). For eggs the optimum survival range was found to be 30-50 ppt, while that for the yolk-sac larvae was 15-25 ppt. TANDLER and SHERMAN (1981) reported high mortality of early larval stages at high salinity (40 ppt). This data on early larval stages, when during the critical period of transition to active feeding (search for the

food and preying) salinity should maintain optimum level of metabolic and physical activity, available so far, are insufficient to be applied in practice.

The objective of our study was to examine the effects of various salinities (5-38 ppt) on survival of gilthead sea bream at constant temperature from hatching to the point of adaptation to active feeding (to an age of 22 days). The study was undertaken to examine the feasibility of the artificial rearing under the conditions along the Adriatic coast.

MATERIAL AND METHODS

Yolk-sac larvae and older larvae used in this study originated from induced spawning of broodstock raised from juveniles which were caught in the Kaštela Bay and in the Neretva River estuary three to four years earlier. Fish were maintained in aquaria at ambient temperature (10.3-25.8 °C) and salinity (35.6-38.7 ppt), and fed daily with sardine and mussel.

Maturation of five females was induced by injection of human chorionic gonadotropin. Fertilization of eggs of each female was achieved using milt stripped from two males. Dry fertilization lasted for 8 min. The remaining spermatozoa were rinsed through a 350-μm sieve with a light spout of fresh sea water and eggs were transferred to glass jars. Ten minutes later fertilized eggs floating on the surface were collected and transferred to incubation tank in density of approximately 100 eggs/L. The incubation temperature and salinity were constant (14.0±0.21 °C and 38 ppt) and the same as in the broodstock tank. Streptomycin sulfate (30mg/L) was added at the beginning of incubation to control bacterial contamination. Water renewal occurred theoretically every 48 hours.

Dead eggs were removed from the tank bottom at day 1 and 2 after fertilization. Five days after fertilization hatching occurred. Hundred yolk-sac larvae were placed in each cylinder containing different volume of filtered sea water (38 ppt). Adding distilled water in drops (from 1 to 4 hours) salinity was diluted to seven different values. Salinities lower than 38 ppt (ambient) were controlled every day by adding distilled water. Despite the relatively low stocking density (1 individual per 20 ml) slight aeration was provided from the cylinder bottom. The trials took place in stagnant sea water under artificial light (approx. 800-1200 lux) with 12-12 photoperiod. Glass jars were placed in a water bath of temperature as that of the ambient of Kaštela Bay marine environment. A very low water flow was allowed through the bath. Sea water temperature was measured twice daily (at 7 a.m. and 2 p.m.) at which time yolk-sac larvae were counted and the dead larvae removed.

Statistically, comparisons were made using the G-test of independent samples at three-day intervals for survival of larvae (SOKAL and ROHLF, 1969). G-test is based on the distribution of log likelihood distribution or G-distribution. The distribution

$$G = 2 \ln p/p,$$

where p and p are observed and postulated probability can be approximated by χ^2-distribution when samples are large. The employment of G-test does not require any special mathematical sophistication so that this method has considerable advantages over the conventional χ^2-test. The computational formula for the G-statistics can be rewritten in various ways depending upon the particular application. Frequently used formula for tests of independence is

$$G = 2 \left[\Sigma \, f_i \ln f_i - \Sigma \, f_i \ln p_i - n \ln n \right]$$

The analysis of variance was used for statistically comparisons of the temperatures (SOKAL and ROHLF, 1969). The arithmetical means of survival at different salinities were approximated by fourth order polynomials (SOKAL and ROHLF, 1969).

Experiment 1.

Yolk-sac larvae were held at seven salinities: 5, 10, 15, 20, 25, 30 and 38 ppt. Each experiment was performed in two replicates.

The first group of larvae was starved to avoid any effect of food (e.g. growth and metabolism) on salinity response. Mean daily temperature during the experiment was 12.4±0.15 °C.

Cardiac contractions were measured under binocular microscope by a stop watch, 24 and 48 hours after stocking. Two larvae from each treatment were placed in Petry-dish and heart beats were counted under artificial light (approx. 1000 lux).

The second group of yolk-sac larvae were fed on rotifers (*Brachionus plicatilis* ; 7-9 individuals/ml) which were sieved through a 150 μm net before feeding. The presence of food in guts of larvae was observed. Rotifers were fed on unicellular green algae (*Chlorella* sp.) and on diluted baker yeast (20-25%). Mean daily temperature in the culture jars was maintained at 12.6±0.72 °C.

Experiment 2.

Four of the investigated salinities (15, 20, 25 and 30 ppt) in the Experiment 1. gave optimum survival of gilthead sea bream larvae showing no significant differences. Testing at these salinities was repeated in the Experiment 2. to prove that there is no difference between their effects on survival or to select any of them as the most optimum. The ambient salinity (38 ppt) was tested too. Experimental procedure was the same as in the Experiment 1, but without replicates. During the experiment, mean sea water temperature was 13.1±0.67 °C.

RESULTS

Experiment 1 .

Survival of starved gilthead sea bream yolk-sac larvae at different salinities (5, 10, 15, 20, 25, 30 and 38 ppt) is given in Fig. 1A.

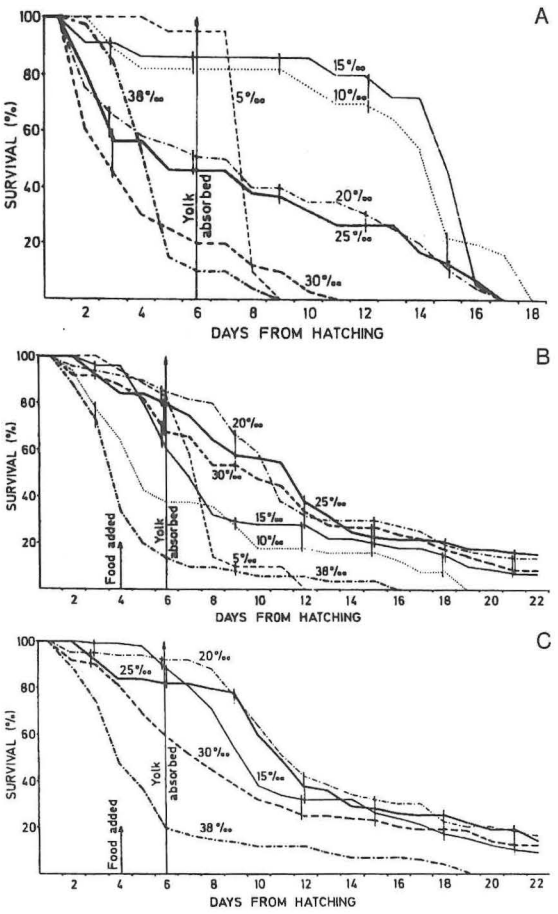

Fig. 1. Means of survival of gilthead sea bream (*Sparus aurata* L.) larvae at different salinities and constant temperature. (A) starving larvae, (B) and (C) fed on rotifers. Vertical lines connect salinities between which G-test showed no significant (P<0.05) difference in survival rate. Curves (A and B) represent the mean of two replicates.

All larvae died at 5, 30, and 38 ppt between the days 9 and 11, and at 10, 15, 20 and 25 ppt on days 17 and 18.

Vertical lines (in Fig.1A) connecting various salinities show no significant differences (P<0.05) in survival between 10 and 15 ppt salinities (by the day 12 after hatching) and between 20 and 25 ppt (by the end of the experiment when all starving larvae died).

By yolk sac resorption (about 130 hrs) larvae showed good survival at 5, 10 and 15 ppt salinities (95, 82 and 86%), somewhat poorer survival at 20 and 25 ppt (51 and 46% respectively) and poorest survival at 30 and 38 ppt (20 and 10% respectively).

Mass mortality of yolk-sac larvae was recorded at 30 and 38 ppt salinities for the first five days after hatching, and at 5 ppt at yolk sac resorption. At salinities of 20 and 25 ppt, yolk-sac larvae showed uniform mortality rate between 0 and 10% daily, with the exception of the days 2 and 3 (35 and 44%). Increased mortality began on days 13-15 and 14-16 at 10 and 15 ppt salinities (43 and 67% respectively).

The heart rate was relatively higher at the higher salinities. At 5, 10 and 15 ppt the heart rate was between 78 and 83 beats/min, with lowest value at 10 ppt. At 20 and 25 ppt it was 92-98 reaching 100-104 beats per minute at 30 and 38 ppt (Fig. 2).

Mean survival at various salinities, calculated form daily survival percentages for the duration of the experiment, was approximated by the fourth order polynomials (Fig. 3A). Curve shows the mean survival of starving yolk-sac larvae to exceed 40% at salinity range of 5 to 20 ppt, with a maximum recorded at approx. 11 ppt salinity.

Survival of yolk-sac larvae and larvae of sea bream in period of initial feeding, fed rotifers (*B. plicatilis*) at reduced salinities and constant temperature (12.6±0.72 °C) is shown in Fig. 1B. There was no significant difference between replicates (ANOVA, $P<0.05$) so we followed the mean of both replicates. Yolk-sac larvae showed good survival at yolk sac resorption, and adaptation to active feeding at 15, 20, 25 and 30 ppt salinities.

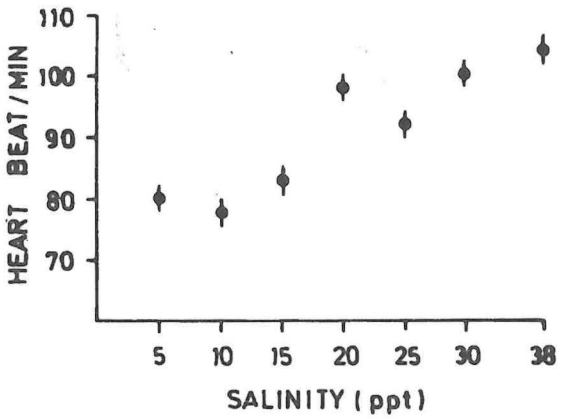

Fig. 2. Mean heart rate (beat/min) with standard deviation, of gilthead sea bream (*Sparus aurata* L.) larvae at different salinities.

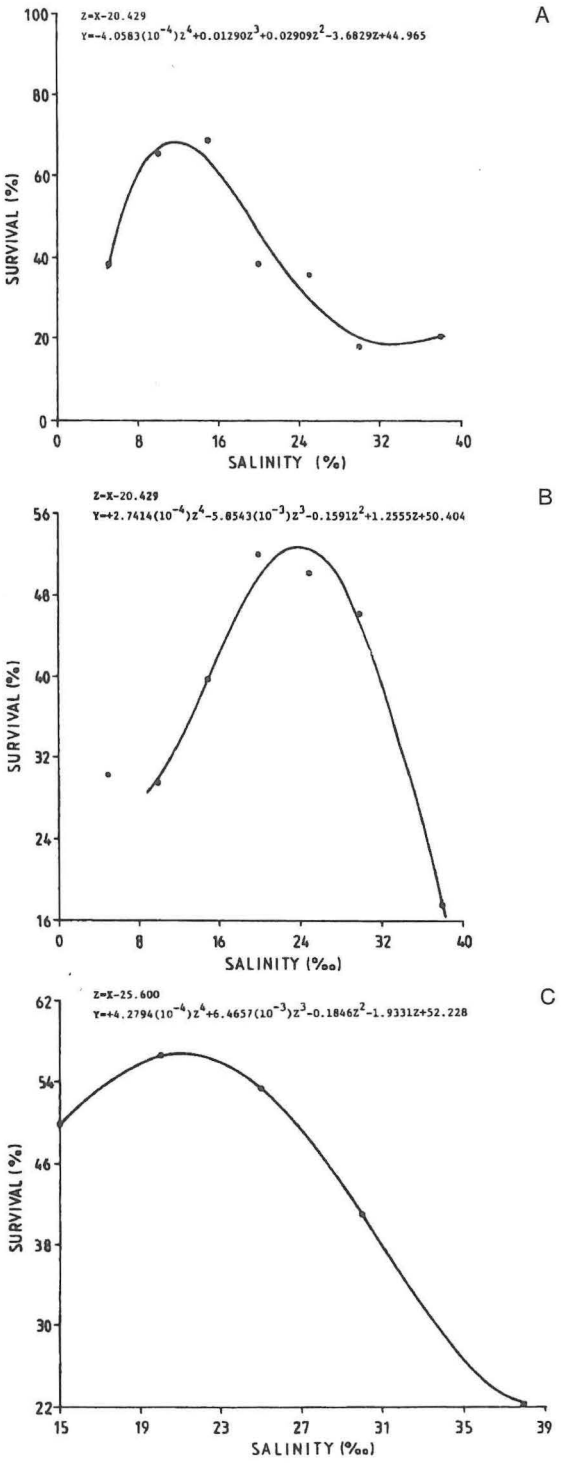

Fig. 3. Survival rate of gilthead sea bream (*Sparus aurata* L.) larvae, calculated as mean of daily survival percents at different salinities for the duration of the experiment, and approximated by fourth order polynomials. (A) starving larvae (B) and (C) fed on rotifers. Curves (A and B) represent the mean of two replicates.

In the fed group the G-test showed a statistically significant correlation ($P<0.05$) between survival at different salinities. Survival in extreme salinities, 5, 38 and to a certain extent 10 ppt, differed statistically from that in the rest of salinities (15, 20, 25, 30 and to a certain extent from 10 ppt). No significant difference ($P<0.05$) was detected between 15, 20, 25 and 30 ppt from day 12 (when almost all the larvae started to feed actively) to day 22. This is indicative of an optimum survival of early larval stages at these salinities (Fig. 1B).

By the time of yolk sac resorption (about 130 hours) survival of 83, 85 and 80% was detected at salinities of 5, 20 and 25 ppt respectively. Survival was slightly poorer at 15 and 30 ppt (60 and 68% respectively) and poorest at 10 and 38 ppt (not exceeding 38 and 14% respectively).

Mass mortality occurred before complete yolk sac resorption at 10 and 38 ppt salinities and during transition to active feeding at 5 and 15 ppt. No sudden larval mortality was recorded at 20, 25 and 30 ppt. All larvae died on day 12 at 5 ppt, day 16 at 38 ppt and day 19 at 10 ppt. At the end of the experiment good survival was detected at 15 and 30 ppt. It was greater at 20 and 25 ppt (14 and 16 % respectively) (Fig. 1B).

Stomachs of larvae that died at 5 ppt salinity were empty, while some traces of food were found in a few larvae that died at 38 ppt. Stomachs of 12% larvae which had completely absorbed yolk and died at 10 ppt also contained food.

Mean survival at various salinities of two replicate trials of yolk-sac larvae and larvae fed rotifers calculated from daily survival percentages for the duration of the experiment, were approximated by the fourth order polynomial (Fig. 3B). Curve of the fourth order polynomial best fitted to survival of larvae fed rotifers at salinities between 15 and 30 ppt.

Experiment 2

Fig. 1C depicts survival of gilthead sea bream from hatching to day 22 at 15, 20, 25, 30 ppt (salinities which proved satisfactory for survival and active feeding). Significantly ($P<0.05$) poorer survival rates were recorded at 30 and 38 ppt during the period from yolk-sac larvae to active feeding (day 3 to day 12). The best survival of yolk-sac larvae by the time of yolk sac resorption occurred at 15, 20 and 25 ppt. Survival was slightly lover at 30 ppt and lowest at ambient salinity. High survival rate at these salinities was recorded several days after the yolk was resorbed, followed by a period of sudden mortality so that 32 to 42% of larvae survived by day 12.

A rather short period of mass mortality of early stages during active feeding appeared at all salinities. It occurred before yolk sac was absorbed at 38 ppt, during the yolk sac absorption and the beginning of active feeding at 30 ppt (between day 4 and 10) and after yolk sac absorption and initial active feeding (between day 8 and 12) at 15, 20, and 25 ppt salinities.

From day 15 to the end of experiment no significant difference in survival of gilthed sea bream larvae between 15, 20, 25 and 30 ppt salinities was recorded (Fig. 1C).

Mean survival of gilthead sea bream larvae at various salinities, calculated from daily survival percentages for the duration of the experiment, were approximated by the fourth order polynomials (Fig. 3C). The curve shows good survival rates at 15-30 ppt salinities.

DISCUSSION

Following the results of FREDDI *et al.* (1981), who found the optimum survival range from 30-50 ppt, eggs of gilthead sea bream in our experiment were incubated at ambient salinity (38 ppt, at which broodstock conditioning and spawning were performed). At salinities higher than approximately 30 ppt live eggs float at the surface whereas the dead ones fall to the bottom and may be easily removed. Since live eggs do not mix with the dead eggs, they are not exposed to high bacterial and fungal contamination what is of high practical importance.

After transferring the newly hatched larvae to experimental jars salinity was reduced very fast so that, presumably, this shock affect-

ed the survival for the first few days of the experiment (ALDERDICE *et al.*, 1979). However, no sudden larval mortality was recorded in this period at lower salinities.

Highest survival of starved yolk-sac larvae of gilthead sea bream (exceeding 80%) by the time of yolk sac absorption was recorded in our experiment at lowest salinities, 5, 10 and 15 ppt. With starved larvae we wished to avoid any effect of food and food intake on growth and metabolism (KINNE, 1960). Beneficial effect of lower salinities to teleosts larvae HOLLIDAY (1969) attributed to less osmotic labor performed. FREDDI *et al.* (1981) investigated survival of gilthead sea bream yolk-sac larvae on salinities from 15 - 45 ppt. Eggs were incubated at 35 ppt and 18 °C. The optimum survival range for the yolk-sac larvae was found to be 15-25 ppt. WOO and MURAT (1981) verified the hypothesis that survival of the red sea bream (*Chrysophrys major*) larvae during starvation is highest in an iso-osmotic medium. TANDLER and SHERMAN (1981) grew the gilthead sea bream yolk-sac larvae at 40 ppt and obtained survival not exceeding 5% until day 12 after hatching. However, on day 12 survival of 6-12% was obtained at 38 ppt in our experiment. ALDERDICE *et al.* (1979) suggested that maximum salinity tolerance of early Pacific herring larvae is likely to be found in relation to salinities associated with minimum osmotic work.

Energy expenditure for consuming yolk reserves is mainly limited to basal metabolism, since yolk-sac larvae are in general non mobile. Energy is expended on continuing organogenesis and osmotic regulation of body fluids through the skin, since newly hatched yolk-sac larvae may have no gill filaments and a kidney represented only by a pronephric glomerulus (HOLLIDAY, 1969). So it may be assumed that the level of metabolism is closely related to the intensity of osmoregulatory mechanism. The level of metabolism at varying salinities may be indicated by the rate of heart beats. As established in our experiment heart rate in gilthead sea bream larvae relatively increased at higher salinities. The dependence of cardiac contraction rate on salinity was studied in some other teleost larvae. For example, KRYZHANOVSKY (1956) found that the heartbeat rate of *Clupea harengus membras* was less rapid at salinity of 4-5 ppt than at 25 ppt. HOLLIDAY and BLAXTER (1960) recorded a reduced rate of heart beat in yolk-sac larvae of *Clupea harengus* transferred from high to low salinity.

Gilthead sea bream larvae fed rotifers showed best survival at salinity range from 15 to 30 ppt. Between days 10 to 22 in both our experiments (when transition to active feeding affected survival) no significant difference in survival was detected between 15, 20, 25 and 30 ppt salinities. The second experiment was carried out to confirm this result.

KATAVIĆ (1984) studied larval survival in gilthead sea bream fed copepod nauplii *Tisbe furcata* and rotifers at 20, 30, 33 and 37 ppt salinities and 19 °C. Since mass mortality of larvae occurred at 30, 33 and 37 ppt by day 10, all experiments except that at 20 ppt were discontinued. By day 60, 13% of the larvae survived.

Both experiments suggested better survival in this period in slightly hypertonic media. We consider this period as very important, especially in commercial rearing since it is the initial point of interaction between three parallel chains in the production of gilthead sea bream fingerlings (phytoplankton, zooplankton, fish), so that any mortality of greater extent produces serious commercial losses.

Since larvae in our first experiment were not fed, they rapidly consumed the rest of energy reserves in a hypertonic medium. In reduced salinities (approximately isotonic media) the consumption of reserves would be slower (at the same temperature) owing to the lower metabolism rate and reduced activity. This would result in greater survival of starved larvae. However, such reduced physical and metabolic activity in fed larvae at the time of transition to active feeding very likely leads to quite the contrary effect: poor survival due to inefficiency in prey capture. To confirm this statement further studies are called for. Still the experiments on yolk-sac larvae alone, that is on starving larvae should be carefully applied in the production process.

These experiments determined that salinity range of 15-30 ppt makes possible an optimum physical and metabolic activity of gilthead sea bream larvae upon yolk sac resorption at the time of transition to active feeding. Taking into account the results of FREDDI *et al.* (1981), who obtained the highest percentage of hatched larvae upon incubation in a hypertonic medium (30-50 ppt at 18 °C), as well as those of ALDERDICE *et al.* (1979) on the influence of the incubation salinity on larval performance, we would like to suggest that the salinity of about 30 ppt is optimum for the process of production of gilthead sea bream fingerlings, from the beginning of incubation to the end of adaptation to active feeding. This may be helpful in avoiding the shock consequences due to eventual reduction or raise of salinity during critical period of larval development, that is transition to active feeding.

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Preživljavanje ličinačkih i ranih poslijeličinačkih stadija komarče (*Sparus aurata* L.) na različitim vrijednostima saliniteta

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KRATKI SADRŽAJ

Ispitivani su utjecaji sedam različitih vrijednosti saliniteta (5, 10, 15, 20, 25, 30 i 38 ppt) na preživljavanje ličinačkih i ranih poslijeličinačkih stadija komarče (*Sparus aurata*) od izvaljivanja do 22 dana uzgoja, pri stalnoj temperaturi. Postavljena su dva pokusa. U prvom su tretirane ličinke u gladovanju i hranjene rotatorijama (od 5 do 38 ppt), a u drugom samo hranjene rotatorijama (od 15 do 30 ppt). Bolje preživljavanje (od 36 do 68 %) dobijeno je na sniženim vrijednostima saliniteta (5, 10, 15, 20 i 25 ppt) kod ličinki u gladovanju, a slično (od 43 do 54 %) kod hranjenih ličinki (15, 20, 25 i 30 ppt). Na ambijentalnom salinitetu (38 ppt) i vrlo niskim salinitetima (5 i 10 ppt) preživljavanje hranjenih ličinki ne prelazi 20%. Vrijednosti aritmetičkih srednjaka preživljavanja na ispitivanim salinitetima procijenjene su polinomom IV reda.