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The sinergetic effect of temperature and salinity on Rotifer *Brachionus plicatilis* (O. F. MÜLLER) population growth and lorica size in mass rearing

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The synergetic effect of temperature (22, 27,35°C) and salinity (20,27,38 psu) on population growth and changes in lorica length and width of the rotifer Brachionus plicatilis *was investigated under mass rearing conditions. The greatest average population density (432.4 ind. ml^{-1}) and the fastest doubling time (D=17.2) was noted at a salinity of 20 psu and at 22°C. Daily harvesting 50-60% of the contents of one 400 l volume tank, satisfied the food intake of 0.5-0.6 x 10^6 reared fish larvae. Various salinity and temperature combinations resulted in significantly different lorica sizes (ANOVA, P<0.001). This shows that it is possible to produce rotifers with the required lorica size, depending on the functional mouth gape of larvae. Differences noted in the growth rates of rotifer numbers and the variety in lorica size in mass rearing conditions was attributed to the phenomena of cyclomorphosis, stimulated by the synergetic effect of salinity and temperature. This is confirmed by the increase in lorica dimensions after equalizing conditions in the experimental tanks with those in the brood stock.*

Key words: *Brachionus plicatilis*, lorica size, mass rearing, salinity, temperature.

INTRODUCTION

Numerous data exist on the influence of temperature and salinity on population growth of *Brachionus plicatilis*. Many of the research performed on this species show how rotifer populations multiply faster when bred under higher temperature and lower salinity. However, the food intake process of fish larvae is a relatively complex problem and does not depend only upon the quantity and quality of available food, but also on its size. Prey size is a function of mouth opening size (ARTHUR, 1976; BEYER, 1980) and rotifer size should not exceed 38% of the maximum mouth opening size of larva (HUNTER, 1980; YUFERA, 1982 and POLO *et al.*, 1992).

In the first days of exogenous food intake, fish larvae select the smallest rotifers, and the prey size increases with the growth of larvae and their mouth openings (HUNTER, 1980). HELPS (1982) presents the fact that the larvae of *Sparus aurata* show a clear discrimination between smaller and larger rotifers during feeding, and that smaller larvae (until 85 hours after hatching) preferred rotifers 30-70 m wide, and avoided those 90-100 m wide. In older larvae (160 hours after hatching), the choice of rotifers

goes initially to small rotifers (191 μm x 138 μm) followed by larger ones (258 μm x 171 μm) when the fish is 6 days old (LASKER *et al.*, 1970; HUNTER and KIMBRELL, 1980).

With the increase in number of reared marine fish species of various larval size and mouth openings, there is an increased need for the rearing of rotifers of various body sizes. FUKUSHO and IWAMOTO (1980) in the outdoor mass rearing of rotifers registered cyclical changes in the lorica size. In warmer months, when temperatures are high, smaller (S-type) rotifers with lorica lengths of 205 - 304 μm are dominant, while larger (L-type) rotifers with lorica lengths of 306 - 347 μm are dominant in the winter months. The authors contributed the differences in rotifer size to cyclomorphosism. SERRA and MIRACLE (1983) also attribute the appearance of variously sized rotifers found in Spanish lagoons and those reared in the laboratory to cyclomorphosis. The same authors are of the opinion that alometric coefficiencies are influenced by environmental factors, mostly by temperature and salinity, whereas, OKAUCHI and FUKUSHO (1985) believe L-type and S-type rotifers to be genetically different. YUFERA (1982) and SNELL and CARRILLO (1984) indicate that the lorica length of *Brachionus plicatilis* is genetically determined. By changing food and salinity concentrations, it is possible to somewhat alter the modification ratio in reared populations of small and large rotifers. The effects of temperature on rotifer size can be attributed to species characteristics. SNELL and CARRILLO (1984) discovered that size of *Brachionus plicatilis* was affected by the food; smaller rotifers were obtained when fed with baker's yeast and larger when fed with the phytoplanktonic algae *Schizothrix sp.*

Faced with the problem of rearing enough quantities of adequately sized rotifer, we decided to investigate the synergetic effect of various temperature and salinity combinations on population growth, as well as on the length and width of lorica of *Brachionus plicatilis* in mass rearing.

MATERIAL AND METHODS

Rotifers

The rotifer *Brachionus plicatilis* culture originates from the National Center for Mariculture, Eilat, Israel (KATAVIĆ, 1984), and was maintained in our laboratories since 1982. The stock culture is maintained in a thermostatic chamber at a temperature of 21 ± 1°C, at a seawater salinity of approximately 38 psu, at photoperiods of 12 L: 12 D, and is fed with green microalgae (*Nannochloropsis sp.*). Prior to the experiment, rotifers in the stock culture had loricas averaging 162 ± 37 μm (range 98-242 μm) in length and averaging 113 26 μm (range 52-178 μm) in width. In this population, 44% of the rotifers had lorica lengths below 150 μm and 56% from 150 to 250 μm. 6% of the rotifers had widths below 80 m, 80% from 80 to 150 μm and 14% were over 150 μm.

Seawater and freshwater

The South Adriatic seawater (SASW) used in the experiment had a salinity of 38 psu and a temperature 16 - 17°C. SASW was pumped from depth of 8 m, 30 m off the coast, into a 10 m high gravitational tank, and supplied, by gravity, to the aquarium tanks. Prior to its use, the water was filtered through three mechanical filters (10, 5 and 1 μm) and was sterilized by means of a flow-through UV lamp. The SASW was diluted with freshwater to a salinity of 38, 27 and 22 psu.

Food intake

The green microalgae *Nanochloropsis* sp. was used as rotifer food. It was reared up to concentration of 25-60 x 10^6 cells ml^{-1}, and we maintained a constant concentration of algae of 4 x 10^6 cells ml^{-1} in the rotifer rearing tank.

Experimental design and conditions

We used three plastic tanks containing 400 l each, with aeration system of our own design

(SKARAMUCA, 1994). The medium was heated by tubular, ceramic 2KW heaters reaching from top to bottom.

In the prepared rearing medium, we inoculated rotifers at an average concentration of 10 ind. ml^{-1} (range 8-11 ind. ml^{-1}). To avoid stress, temperatures were gradually raised from 22°C to 27°C over a 24 hour period, and to 35°C over the following 24 hours. The same procedure was used in lowering salinity. Salinity from 34 to 27 psu were reduced over a 24 hour period and to 20 psu over 48 hours, according to the procedures described by FUKUSHO and IWAMOTO (1980) and LUBZENS *et al.* (1984a, 1984b).

Control of abiotic factors, rotifer count, food conditions and lorica length and width measurements

Period of first 5 days covers the time to adjust to rearing conditions and to the changeover of at least two generations of rotifers. Measurements of temperature, salinity and DO were performed on a daily basis (from the first to the tenth day) at around 9:00 am. Food density and number of rotifers per ml in each tank were also controlled daily. Following the period of acclimation (from the sixth to the tenth day), we measured the lorica lengths and widths of 50 rotifers from each container. Food density expressed as No. cells ml^{-1} were counted in the BRKER-TRK chamber.

5 samples were retrieved daily from each tank using a 1 ml pipette for rotifers counts. The length and width of loricas were measured using the WILD-HEERBRUGG (type 325400) microscope, equipped with WILD-HEERBRUGG MMS 235 measuring system.

Statistical analysis

Data were subjected to statistical analysis using the analysis of variance (ANOVA) and WILCOXON singed rank test. Statistical analysis was performed using a STATISTICA software package.

RESULTS

The effects of various temperature and salinity combinations on population density growth

In tanks with a salinity of 38 psu, the increase in number of rotifers is shown in Fig. 1 for all rearing temperatures, with the highest values obtained on the tenth day.

A maximal number of 249.4 ind.ml^{-1} was achieved at day 10 at temperatures of 35°C. The coefficiencies of variation among 5-replicate samples were relatively low: at 35°C, CV=16.1 ± 9.9; at 27°C, CV=12.6 ± 6.0; at 22°C, CV=15.3 ± 6.6. The highest average growth coefficient (R = 0.020 ± 0.003), as well as the lowest doubling time (D = 35.3±12.9) were also noted at a temperature of 35°C.

In tanks with a salinity of 27 psu, the variations in rotifer density values were bigger (Fig. 2). The highest average individual number at 374.6 ind. ml^{-1} was noted on the ninth day at a temperature of 35°C. In the remaining tanks, highest average rotifer numbers were determined on the day 10, 307.4 ind. ml^{-1} at temperatures of 27°C and 204.6 ind. ml^{-1} at temperatures of 22°C. The coefficiencies of variation were low: at 35°C, CV=11.5 ± 14.8; at 27°C, CV=6.3 ± 3.3; at 22°C, CV=9.7 ± 6.2. The highest average growth coefficient (R=0.024 ± 0.010) and the lowest doubling time (D=32.1 ± 11.4) were also noted at temperatures of 35°C.

In tanks with a salinity of 20 psu, the maximum average number of rotifers at 405.0 ind.ml^{-1} was achieved on the seventh day in the first tank (Fig. 3). In the second tank, the highest average value at 409.6 ind.ml^{-1} was also noted on the seventh day, and in the third tank on the tenth day, 423.2 ind.ml^{-1}. The coefficiencies of variation were low: at 35°C, CV=9.6 ± 7.3; at 27°C, CV=9.1 ± 4.8; at 22°C, CV=6.8 ± 4.7. The highest average growth coefficient (R=0.015 ± 0.012) was noted at temperatures of 35°C, while the lowest doubling time (D=17.2 ± 97.5) was recorded at temperatures of 22°C.

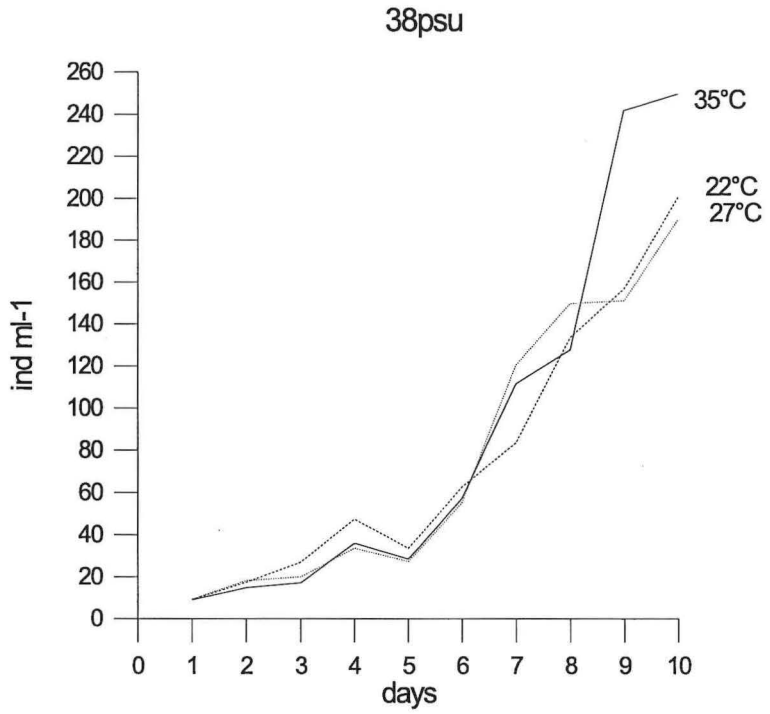

Fig. 1. Values in individual rotifer numbers at various combinations of temperature and salinity 38 psu during 10 days (day1-values in individual rotifer numbers inoculated at the start of the experiment)

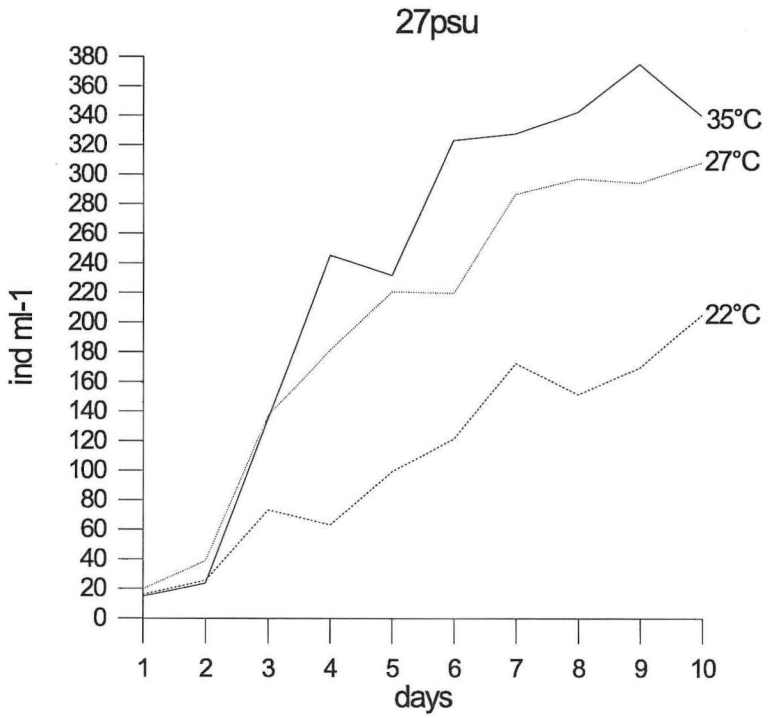

Fig. 2. Values in individual rotifer numbers at various combinations of temperature and salinity 27 psu during 10 days (day1-values in individual rotifer numbers inoculated at the start of the experiment)

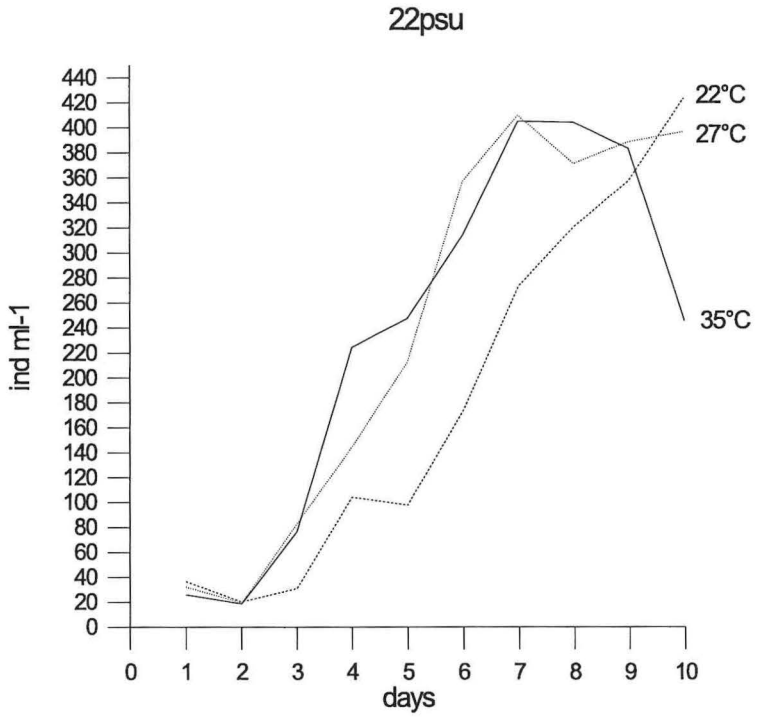

Fig. 3. Values in individual rotifer numbers at various combinations of temperature and salinity 20 psu during 10 days (day1-values in individual rotifer numbers inoculated at the start of the experiment)

The effect of various temperature and salinity combinations on changes in lorica lengths and widths

Significant differences were obtained (ANOVA, F=46.757, P<0.001) by comparing total values of lorica lengths for rotifers reared under various temperatures and salinity. The WILCOXON singed rank test determined significant differences between individual combinations (Table 1). The lengths obtained in rearing at S 38 psu and T 27°C have the lowest average values and significantly differ from all the other combinations (<0.001). Under these conditions, even 88 % of individuals had a lorica length smaller than 150 μm (Table 3). The highest average lorica lengths were measured at a salinity of 20 psu and significantly differ (<0.001) from other combinations except for the culture at 27 psu and 22°C (Table 1).

As a rule, the highest number of individuals (50%) with lorica lengths smaller than 150 μm were found in tanks with a salinity of 38 psu. The highest number of individuals with lorica lengths in the size range 150-250 μm were obtained at salinity of 27 psu, and sizes of more than 250 μm were at salinity of 20 psu (Table 3).

Significant differences were also obtained (ANOVA, F=51.961, P<0.001) by comparing length and width of rotifers reared in various salinity/temperature combinations, especially between values obtained at salinity of 38 psu and 20 psu independent of temperature (Table 2). The lowest average widths, and the highest percentage of individuals with lorica width smaller than 80 μm were noted at 38 psu and 27°C (Table 3). The highest percentage of individuals with lorica width between 80-150 μm were obtained with a salinity of 20 psu and 35°C, whereas the combination of salinity of 20 psu and a temperature of 22°C showed the highest percentage of individuals with lorica width greater than 150 μm (Table 3).

Table 1. WILCOXON signed ranks test between values in rotifer length obtained at various combinations of temperature and salinity (n=700; sign. level<0.005; n.s.=non-significant)

	S38T27	S38T22	S27T35	S27T27	S27T22	S20T35	S20T27	S20T22	X±Std
T35	0.001	0.002	n.s.	n.s.	<0.001	<0.001	0.003	<0.001	0.131±0.045
T27		<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.120±0.037
T22			<0.001	n.s.	<0.001	n.s.	n.s.	<0.001	0.136±0.041
T35				0.003	<0.001	<0.001	<0.001	<0.001	0.128±0.043
T27					<0.001	n.s.	n.s.	<0.001	0.136±0.051
T22						n.s.	<0.001	0.264	0.151±0.049
T35							n.s.	<0.001	0.141±0.044
T27								<0.001	0.139±0.056
T22									0.158±0.091

Table 2. WILCOXON signed ranks test between values in rotifer width obtained at various combinations of temperature and salinity (n=700; sign. level<0.005; n.s.=non-significant)

	S38T27	S38T22	S27T35	S27T27	S27T22	S20T35	S20T27	S20T22	X±Std
S38T35	0.004	n.s.	n.s.	n.s.	<0.001	<0.001	<0.001	<0.001	0.083±0.030
S38T27		n.s.	n.s.	n.s.	<0.001	<0.001	<0.001	<0.001	0.079±0.028
S38T22			n.s.	n.s.	<0.001	<0.001	<0.001	<0.001	0.082±0.029
S27T35				n.s.	<0.001	<0.001	<0.001	<0.001	0.079±0.025
S27T27					n.s.	<0.001	<0.001	<0.001	0.084±0.028
S27T22						n.s.	n.s.	<0.001	0.091±0.032
S20T35							<0.001	n.s.	0.096±0.030
S20T27								<0.001	0.088±0.028
S20T22									0.104±0.034

Table 3. Average percentages (%) in numbers of individual rotifers of variously sized length and width categories depending on temperature and salinity combinations

	LENGTH (µm)			WIDTH (µm)		
20 psu	<150	150-250	>250	<80	80-150	>150
35 °C	48.87	45.06	6.07	22.30	63.22	13.48
27 °C	53.02	44.13	2.84	33.17	58.25	8.58
22 °C	36.81	53.87	9.32	19.16	55.03	25.81

	LENGTH (µm)			WIDTH (µm)		
27 psu	<150	150-250	>250	<80	80-150	>150
35 °C	56.34	43.52	0.14	43.48	52.27	4.25
27 °C	50.79	47.50	1.71	41.07	50.81	8.12
22 °C	36.29	58.43	5.28	33.09	52.79	14.12

	LENGTH (µm)			WIDTH (µm)		
38 psu	<150	150-250	>250	<80	80-150	>150
35 °C	85.20	14.80	0	71.60	24.80	0
27 °C	89.50	10.50	0	82.90	17.10	0
22 °C	52.43	45.86	1.71	45.43	46.71	7.86

At the end of our experiment in the tank with 38 psu and 27°C , we continued observing changes in lorica lengths and widths, dependent on the cooling of the rearing medium. Changes were noted immediately after 24 hours (average length 162±39 μm and width 95±21 μm). In the following days, we noted few changes in the average lengths and widths of lorica.

The highest average density of 432.4 ind. ml^{-1} was noted at a salinity of 20 psu and at a temperature of 22°C, with the doubling time (D = 17.2). Similar results were achieved by other authors, who recommend rearing rotifers at lower salinity, 20-25 psu, in order to achieve quicker growth and higher population numbers (RUTTNER-KOLISKO, 1969; EPP and WINSTON, 1977; GATESOUPE and LUQUET, 1981; SANKO and SKARAMUCA, 1986). In our rearing, using higher salinity (27 and 38 psu), higher density was achieved using higher temperatures. Temperatures over 23°C stimulate quicker reproduction of individuals (HIRAYAMA and KUSANO, 1972; PASCUAL and YUFERA, 1983), while those above 35°C stimulate faster reproduction only in the first two days of rearing (KOŽUL and SKARAMUCA, 1998). The above-mentioned results direct us to the need of determining adequate balances in temperature and salinity, when greater densities of rotifer are needed.

Optimal rearing temperatures depend also on the type of rotifer (SNELL and CARRILLO, 1984). In natural environments (HUTCHINGSON, 1967; SERRA and MIRACLE, 1983) and in rearing conditions (FUKUSHO and IWAMOTO, 1980) at lower temperatures, individuals with larger lorica dimensions are predominant. The above-mentioned authors attribute this rotifer phenomena to cyclomorphosis. The cyclomorphosis phenomena has been confirmed for some Dinoflagelata and Diatomeae, Crustacea, as well as rotatorian genera *Brachionus*, *Keratella*, *Notholca* and *Asplanchna*. Considering that cyclomorphosis has been observed in various and systematically separated groups, we consider this to be the effect of adapting to cycle related changes of specific factors, frequently found in coastal marine waters or in lakes. Contrarily, HIRANO (1987) is of the opinion that change in rotifer size is the result of the higher reproduction of individual types of rotifers at certain temperatures. This means that both L-type and S-type exist in the population, and that L-type rotifers dominate at lower temperatures and S-type at higher temperatures. However, according to the results presented by SNELL and CARRILLO (1984), lorica length is genetically determined for the rotifer *Brachionus plicatilis*. ENDO and MOCHIZUKI (1984) are of the opinion that it is possible to achieve small changes (<15%) in the number of differently sized rotifer through changing the kind of food or salinity. Comparing our results with those found in literature, we can state with certainty that our case deals with small S-type rotifers. In spite of the fact that we reared the same type of rotifer, we noted significant differences (ANOVA, $P<0.001$) in lorica lengths and widths and confirmed that optimal combinations of temperature and salinity are required in rearing rotifer of specific size. In this manner, the number of rotifer with altered lorica lengths (44%), and widths (76.7%) changed significantly with respect to the initial values.

In order for large numbers of fish larvae to survive in the first days of exogenous feeding, it is necessary to have adequate rotifer numbers and sizes. OKAUCHI *et al.* (1980) and KAFUKU and IKENOUE (1983) have calculated that 40000-100000 rotifers are needed for one larva, from the moment it starts to feed until transferring to other food.

Maximum opening is not a measure of the prey size which a larva can swallow, but rather its functional part, which does not surpass 40% of the maximum mouth opening of most fish larvae (KATAVIĆ, 1984; MANEEWONG *et al.*, 1986; JUG-DUJAKOVIĆ and GLAMUZINA, 1988; De CHIECHOMSKY, 1967; HUNTER and KIMBRELL, 1980) and 13% of the maximum opening for Pacific Ocean hake (SUMIDA and MOSER, 1980). Especially small, functional mouth openings (98-124 μm) are found with the dog's teeth *Dentex dentex* and dusky grouper *Epinephelus marginatus* (100-120 μm)

(GLAMUZINA *et al.*, 1989; 1998). From observing food intake using live food in our laboratories, we are of the opinion that with such small larvae, not only is the width of the lorica important, but the length as well, considering that the swallowed prey fills up the intestine and stomach area entirely, which can significantly prolong digestion and delay the intaking of more prey. Therefore, our research results have offered the production of adequately sized food for the specified fish with the smallest functional mouth openings.

Our results show that in mass rearing the rotifer *Brachionus plicatilis* at a salinity of 20 psu and at a temperature of 22°C, we can satisfy the food requirements of 0.5 - 0.6 x 10^6 larvae, by rearing rotifers in 4 tanks at 400 l volumes, with a daily harvest of 50-60% of the numbers of rotifers in one tank. Various combinations of salinity and temperature gave significant differences ($P<0.001$) in lorica dimensions. This permits, under identical technical conditions, the rearing of rotifers with appropriately sized lorica, depending on the functional mouth gape of larvae. In mass rearing, we are inclined to attribute the specified differences in the growth rates of rotifer numbers and the various dimensions of lorica, to the phenomena of cyclomorphosis, stimulated by the synergetic effect of salinity and temperature. This fact is confirmed by the increase in lorica dimensions after having equalized conditions in the experimental tanks once again with those in the stock culture.

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Zajednički utjecaj temperature i slanosti na rast populacije i veličinu lorike kolnjaka *Brachionus plicatilis* (O. F. MÜLLER) u masovnom uzgoju

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SAŽETAK

Proučavan je zajednički učinak temperature (22, 27 i 35°C) i slanosti (20, 27 i 38 psu) na rast populacije i promjene u veličini lorike kolnjaka *Brachionus plicatilis* pri uvjetima masovnog uzgoja. Najveća srednja vrijednost gustoće populacije (432,4 ind ml^{-1}) i najbrže vrijeme udvostručenja populacije(D=17,2 h) zabilježene su na slanosti 20 psu i temperaturi od 22°C. Dnevna žetva od 50-60% sadržaja 400 l tanka zadovoljila je hranidbene potrebe 500-600000 ribljih ličinki. Različite kombinacije temperature i slanosti rezultirale su značajnim razlikama u vrijednosti veličine lorika (ANOVA, $P<0,001$). Ovo pokazuje da je moguće dobiti kolnjake željene veličine lorike, a koja ovisi o dimenzijama usta riblje ličinke. Razlike u koeficijentu rasta i veličini lorike u uvjetima masovnog uzgoja je pripisivana pojavi ciklomorfoze, potaknute zajedničkim učinkom slanosti i temperature. Ovo je potvrđeno porastom vrijednosti veličine lorike nakon izjednačenja uvjeta u pokusnim bazenima s onim u matičnoj populaciji.