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Effects of temperature and salinity on *Menidia beryllina* embryos exposed to terbufos*

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ABSTRACT: Embryos of the inland silverside *Menidia beryllina* were exposed to the organophosphorus pesticide terbufos at 9 combinations of temperature (20, 25 and 30 °C) and salinity (5, 12.5 and 20 ‰). Nominal exposure concentrations were 12.5, 25, 50 and 100 µg terbufos l^{-1} with an acetone and seawater control for each temperature/salinity combination. Test durations were temperature dependent and ranged from 5 to 14 d. Endpoints were embryo survival, hatching and percentage of larvae with normal vertebrae. Embryo survival was significantly ($\alpha = 0.05$) lower in tests conducted at 20 °C for all salinities. Salinity affected survival only at combinations of 20 ‰ and 100 µg terbufos l^{-1} Both temperature and salinity affected the percentage hatch, with the lowest hatching occurring in tests at 20 °C and in tests conducted at 20 ‰. The percentage of larvae with normal vertebrae was significantly ($\alpha = 0.05$) reduced from controls at terbufos concentrations of 25 (7 to 32 %), 50 (44 to 62 %) and 100 µg l^{-1} (58 to 73 %) for the 3 temperatures tested, whereas salinity showed no significant effect. Anomalies in the development of vertebrae occurred across all temperature and salinity combinations, and were observed at concentrations as low as 12.5 µg terbufos l^{-1}

INTRODUCTION

Terbufos (S-[(1,1-Dimethylethyl) thio] methyl] 0,0-diethyl phosphorodithioate) is a widely used organophosphorus compound applied as a soil insecticide and nematicide primarily on corn, sugar beets and grain sorghum for protection against a variety of agricultural pests (U.S. Environmental Protection Agency 1983). As of 1983, over 7.9 million acre-treatments (the number of acres treated multiplied by the number of applications) of terbufos were applied in the USA (Smith 1987).Terbufos has been detected in 444 of 2016 surface-water samples from 55 locations, at a maximum concentration of 2.25 µg l^{-1}, and in 9 of 283 ground-water samples from 261 sources, at a maximum concentration of 3.0 µg l^{-1} (U.S. Environmental Protection Agency 1987).

Studies with aquatic animals are limited to several freshwater invertebrate and fish species. Static 96 h LC_{50}'s for technical grade terbufos range from 7.6 to 15.3 µg l^{-1} for rainbow trout *Salmo gairdneri*, 150 to 390 µg l^{-1} for fathead minnows *Pimephales promelas*, and 1.1 to 2.4 µg l^{-1} for bluegill *Lepomis macrochirus* (Mayer & Ellersieck 1986).

Other organophosphate pesticides with anticholinesterase modes of action have been shown to induce teratogenic responses in developing teleosts. Weis & Weis (1974) reported that embryos of the mummichog *Fundulus heteroclitus* exposed to 10 mg parathion l^{-1} exhibited arrested development and cardiac malformations including tube hearts. In the same study, several mummichog fry developing in 10 mg malathion l^{-1} exhibited scoliosis. Exposure of Atlantic silversides *Menidia menidia* to malathion caused optic anomalies such as unilateral and bilateral microphthalmia, unilateral and bilateral anophthalmia and cyclopia (Weis & Weis 1976). The exposed embryos also showed stunted growth and severe cardiac malformations. Embryos of the Japanese medaka *Oryzias latipes* expressed anomalies in the shape and number of the heart chambers when exposed to 3 mg parathion l^{-1} and 10 mg malathion l^{-1}, and 100 % of the embryos developed straight tube hearts when tested at 9 and 18 mg parathion l^{-1} and 40 mg malathion l^{-1} (Solomon & Weis 1979).

The purpose of our study was to determine if terbufos was teratogenic to developing *Menidia beryllina* em-

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bryos, and if so, what influence combinations of temperature and salinity would have on terbufos teratogenicity.

MATERIALS AND METHODS

Fish. Naturally spawned and fertilized embryos of the inland silverside *Menidia beryllina* were obtained from a population of adults collected from Robinson Point, Blackwater Bay, Florida, USA, and maintained in the laboratory. The laboratory spawning system was described by Middaugh et al. (1986, 1987). Fertilized embryos in the blastula stage (Lagler et al. 1977) were harvested from spawning substrates on days when tests were started.

Exposures. A 3 × 3 × 6 complete factorial design was used consisting of 3 levels of salinity (5, 12.5 and 20 ‰), 3 levels of temperature (20, 25 and 30 °C) and 4 terbufos concentrations (12.5, 25.0, 50.0 and 100 µg l^{-1}) with an acetone and seawater control.

Seawater pumped from Santa Rosa Sound, Florida was prefiltered through 20 µm polypropylene filters and secondarily vacuum filtered to 6 µm. The salinity was adjusted to 5, 12.5 or 20 ‰ using deionized glass-distilled water. The filtrate was then autoclaved in aliquots of 2500 ml.

A 1.0 mg ml^{-1} stock solution was prepared by dissolving terbufos (86.0 % technical grade, American Cyanamid Co., Princeton, NJ) in nanograde acetone. Exposure concentrations were provided by adding the appropriate amount of stock solution to 1120 ml of the sterile seawater to yield the desired exposure concentrations. The acetone concentration was equilibrated across all exposure concentrations. For each exposure concentration, 120 ml of media was used for exposures, with the remaining 1 l analysed for terbufos concentration at the beginning of each test.

The 1 l terbufos samples were extracted twice with 100 ml petroleum ether and concentrated to 1 ml. Extracts were analysed by electron capture gas chromatography utilizing a 182 cm × 2 mm i.d. glass column packed with 2 % SP2100 on 100/120 mesh Supelcoport, at a column temperature of 200 °C. The detector was maintained at 300 °C and quantification was accomplished with a Hewlett-Packard 3357 Lab Automation System*.

The loading and exposure procedures were similar to those described by Middaugh et al. (1988). Borosilicate glass culture tubes 16 × 93 mm (window size 11 × 55 mm) were used as exposure vessels. A single blastula-stage embryo was placed into each of 120 numbered, randomly ordered, culture tubes. The tubes were reordered and 6 ml of clean seawater, acetone-seawater mixture or exposure media was pipetted into 20 respective culture tubes for each concentration or control and sealed with a teflon lined screw cap. Thus, 20 tubes were used for each exposure concentration, and each seawater, and acetone control for all of the 9 salinity-temperature combinations tested. The racked tubes were placed in a horizontal position in an incubator and maintained at the desired temperature ± 1 °C with a 14 h light : 10 h dark photoperiod, and a light intensity of 17.5 µE m^{-2} s^{-1}. Daily, each rack was inverted 5 times to facilitate mixing and to reduce the potential for fungal infections (Hemmer unpubl. data).

Observations. Individual embryos were checked 24 h post-exposure and then monitored daily until the commencement of hatching. Test duration was temperature dependent, ranging from 14 d at 20 °C to 7 d at 25 °C and 5 d at 30 °C. Tubes containing hatched larvae were placed into an ice bath until the cessation of swimming to facilitate observations. A Zeiss Invertoscope D Microscope equipped for photomicrography was used to categorize results. Larvae were scanned for craniofacial and cardiovascular malformations, however, primary emphasis was on observation and quantification of skeletal anomalies. The skeletal deformities were scored using a numerical 'severity-index' (Table 1) which was based on classification schemes devised by Weis & Weis (1977), Weis et al. (1981) and Weis & Weis (1982).

At the end of each test, 5 tubes from each treatment were checked for dissolved oxygen content by direct measurement within each tube using a Lazar DO-166 dissolved oxygen probe. The pH of test solutions in 3 additional tubes were measured from each treatment using an Orion SA 520 pH meter and a Fisher pencil combination electrode. The test solutions were gently stirred during all measurements using 7 × 2 mm magnetic stir bars.

Statistical analysis. For each test, the combined percentage survival of embryos and larvae, the percentage hatch of normally developed larvae, and the percentage of larvae with vertebral anomalies was calculated for each treatment level. All percentages were arcsine transformed before analysis. Percentage combined survival of embryos and larvae, percentage normally developed larvae, and the percentage of larvae with vertebral anomalies were each analysed by a fixed effects, 3-way analysis of variance (ANOVA) with interaction model using SAS Proc GLM procedures (SAS 1985a). Comparisons between treatments and control(s) were performed using the Bonferroni multiple comparison procedure at $\alpha = 0.05$, where appropriate (Neter et al. 1985).

Response surface methodology was used to show the relationship between normally developed larvae and the variables: temperature, salinity and terbufos con-

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Table 1. *Menidia beryllina*. Observed teratological responses in skeletal development of embryos and larvae, with numerical severity-index values assigned to vertebral anomalies

Value	Effect
0	None observed
1	Vertebral anomaly slight, in one location
2	Vertebral anomalies slight, in two locations
3	Vertebral anomaly severe
4	Vertebral anomalies severe, and in two or more locations

centration. The following second order polynomial regression model was used to analyse the data

$$Y = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

where Y = response variable; x_1, x_2, x_3 = measured concentration of terbufos ($\mu g\ l^{-1}$), salinity and temperature, respectively, and b_0 to b_{23} are the estimated regression coeffcients for each term (Box & Draper 1987).

Utilizing the coefficients provided by the multivariate regression model, 5 contour plots were constructed using the experimental levels of temperature and salinity, and data retransformed from arcsine to percentage for normally developed larvae. The 5 plots were created by fixing each of the nominal concentration levels within the model and then plotting each using the SAS Proc G3D procedure (SAS 1985b).

For each temperature level, histograms of the combined percentage survival of embryos and larvae and the percentage of normally developed larvae were produced using SAS Proc GCHART procedures (SAS 1985b).

RESULTS

Nominal and measured concentrations of terbufos at the beginning of each of the 9 tests are listed in Table 2. Recovery was ≥ 90 % with < 15 % variation for all spiked samples analysed. The mean percentage dissolved oxygen (DO) saturation and pH for each test are summarized in Table 3. In all tests, the DO remained above 60 % saturation, with the exception of the test at 20 °C and 5.0 ‰ S in which the measured DO in the 3 tubes at differing terbufos concentrations was below 60 %, but remained above 51 %.

Survival of seawater and acetone controls was greater than 90 % for all combinations of temperature and salinity (Figs. 1A to 3A), except the extreme of 20 °C and 5.0 ‰ where survival was 55 % in the seawater controls and 65 % in the acetone controls (Fig. 1A). Hatching at 25 and 30 °C was 85 % or greater for embryos in seawater and acetone controls across all 3 salinities.

In the test at 20 °C and 5 ‰ S, 3 seawater control embryos and 4 acetone control embryos showed reduced cephalization, malformation of the heart with lack of circulation, and stunting of the skeletal axis. None of these embryos lived to hatching. Two additional acetone control embryos in this test developed vertebral malformations. In this case, 1 hatched embryo showed a single, slight vertebral anomaly, while 3 separate vertebral anomalies were observed in the second hatched embryo.

Hatching of controls at 20 °C with 12.5 and 20 ‰ S remained at 80 % or above (Fig. 1A), but decreased at 5 ‰ to 50 and 55 % for seawater and acetone controls, respectively.

Results of the 3-way ANOVA showed a significant difference ($p = 0.001$) between the 3 temperatures for percentage embryo-larval survival across all combinations of salinity and terbufos concentrations, with survival at 20 °C significantly reduced ($\alpha = 0.05$) from tests conducted at 25 and 30 °C. The analysis also showed a significant interaction ($p = 0.004$) between salinity and terbufos concentration. Only embryos exposed to the combination of 20 ‰ S and 100 µg terbufos l^{-1} showed significantly lower ($\alpha = 0.05$) survival from controls, and also from embryos treated at 5 and 12.5 ‰ S, and 100 µg terbufos l^{-1}.

Analysis of percentage hatch indicated a significant interaction between terbufos concentration and temperature ($p = 0.0045$), and terbufos concentration and salinity ($p = 0.0006$), but not between temperature and salinity ($p = 0.0892$). Figs. 1A, 2A and 3A show the trend toward diminished hatching of embryos with decreas-

Table 2. *Menidia beryllina*. Nominal and measured exposure concentrations (in $\mu g\ l^{-1}$) of terbufos for tests conducted at 20, 25 and 30 °C and 5, 12.5 and 20 ‰ S

Nominal concentration	Measured concentration								
	20 °C			25 °C			30 °C		
	5 ‰	12.5 ‰	20 ‰	5 ‰	12.5 ‰	20 ‰	5 ‰	12.5 ‰	20 ‰
12.5	11.8	9.7	10.6	10.5	9.2	11.1	10.3	10.0	10.0
25.0	20.6	19.7	20.9	21.1	20.4	20.8	20.8	23.2	20.0
50.0	42.9	39.5	42.9	41.9	40.7	42.7	43.1	44.2	30.8
100.0	89.1	84.4	96.1	90.7	80.3	87.7	84.4	91.8	85.2

Table 3. *Menidia beryllina*. Mean pH and mean percentage dissolved oxygen (DO) saturation for tests conducted at 20, 25 and 30 °C and 5, 12.5, and 20 ‰ S

Nominal concentration ($\mu g\ l^{-1}$)	5 ‰				12.5 ‰				20 ‰			
	pH	Range	DO	Range	pH	Range	DO	Range	pH	Range	DO	Range
At 20 °C												
Seawater control	6.9	0.31	80	10	7.4	0.53	93	7	7.5	0.15	98	3
Acetone control	6.6	0.39	64	11	7.0	0.44	87	4	6.9	0.07	83	10
12.5	6.7	0.10	70	9	6.9	0.21	90	12	6.9	0.10	87	30
25.0	6.4	0.30	63	18	7.1	0.11	85	10	7.0	0.02	88	4
50.0	6.6	0.22	69	6	7.1	0.08	82	19	7.0	0.02	83	3
100.0	6.4	0.23	64	11	7.0	0.14	88	4	6.9	0.06	89	7
At 25 °C												
Seawater control	6.9	0.24	91	8	7.7	0.16	97	8	8.2	0.47	89	22
Acetone control	6.6	0.16	91	9	6.8	0.37	95	10	7.2	0.32	83	21
12.5	6.8	0.22	94	7	6.9	0.15	96	10	7.4	0.54	91	7
25.0	6.5	0.23	92	7	7.0	0.05	95	7	7.6	0.76	91	5
50.0	6.4	0.36	89	9	6.7	0.06	97	8	7.5	0.29	86	13
100.0	6.6	0.27	93	6	6.7	0.06	96	7	7.4	0.48	92	5
At 30 °C												
Seawater control	6.8	0.04	89	8	7.4	0.27	99	3	8.0	0.20	80	10
Acetone control	6.4	0.39	83	7	6.7	0.29	95	9	6.9	0.65	81	10
12.5	6.4	0.34	86	3	6.8	0.16	96	5	7.0	0.19	85	5
25.0	6.5	0.21	90	4	6.8	0.16	96	7	7.0	0.13	84	10
50.0	6.3	0.31	88	9	6.8	0.23	97	7	7.1	0.24	78	4
100.0	6.5	0.06	93	6	7.0	0.13	97	6	7.1	0.24	80	3

ing temperature coupled with increasing salinity and increasing terbufos concentration. Hatching was significantly different between the 3 exposure temperatures, and followed a graded response – with the lowest percentage hatch occurring in tests conducted at 20 °C and the highest percentage hatch in tests at 30 °C. The percentage of hatching embryos was also statistically lower ($\alpha = 0.05$) for all tests conducted at 20 ‰ S.

Embryos exposed to terbufos expressed a broad range of developmental malformations. Reduced cephalization occurred in 5.6 % of exposed embryos; primarily in the test conducted at 20 °C and 5 ‰. Microphthalmia was observed in several embryos, but in general its occurrence was sporadic. Cardiovascular anomalies such as defects in the number and structure of heart chambers, and reduced or total lack of circulation, were exhibited in 3.4 % of exposed embryos. Again, the highest frequency of occurrence was in the test conducted at 20 °C and 5 ‰ S.

Skeletal anomalies, in the form of curvature and malformations of the vertebrae, were the most severe and predominant terata observed across all combinations of temperature and salinity. Scoliosis and/or lordosis occurred in 21 % of embryos exposed to terbufos, with 27 % exhibiting malformations in the structure of vertebrae at one or more places along the vertebral column. An example of normally developed vertebrae from a control larvae, and examples of malformed ver-

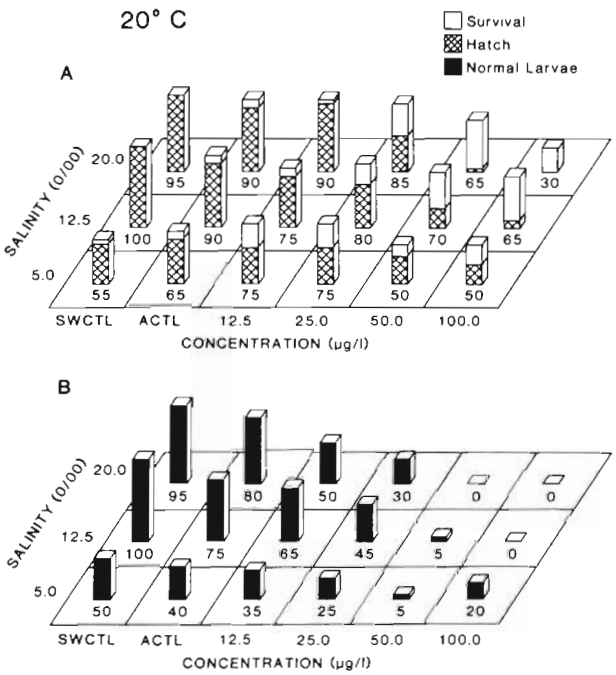

Fig. 1. *Menidia beryllina*. Results of blastula stage embryos exposed to terbufos at 20 °C. (A) Combined percentage survival of embryos and larvae; (B) percentage of normally developed larvae. Symbols are SWCTL: saltwater control; ACTL: acetone control; the number within each block indicates percentage survival and percentage normally developed larvae in A and B, respectively

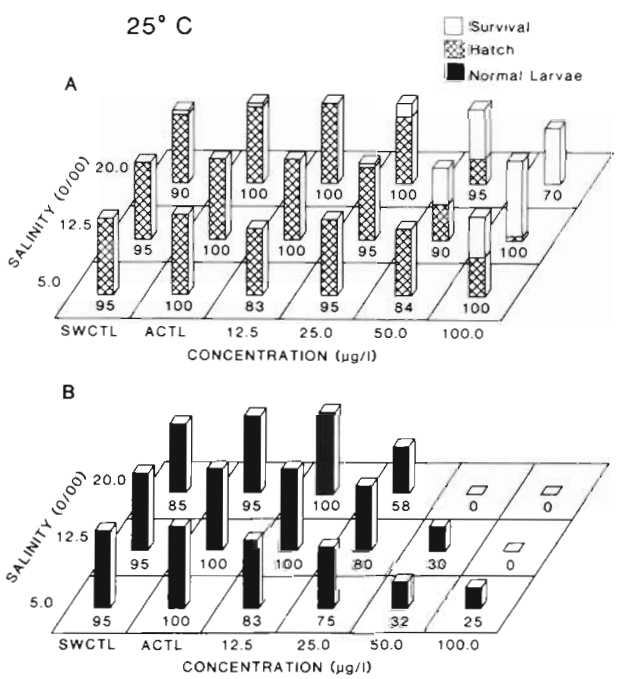

Fig. 2. *Menidia beryllina*. Results of blastula stage embryos exposed to terbufos at 25 °C. See Fig. 1 for further details

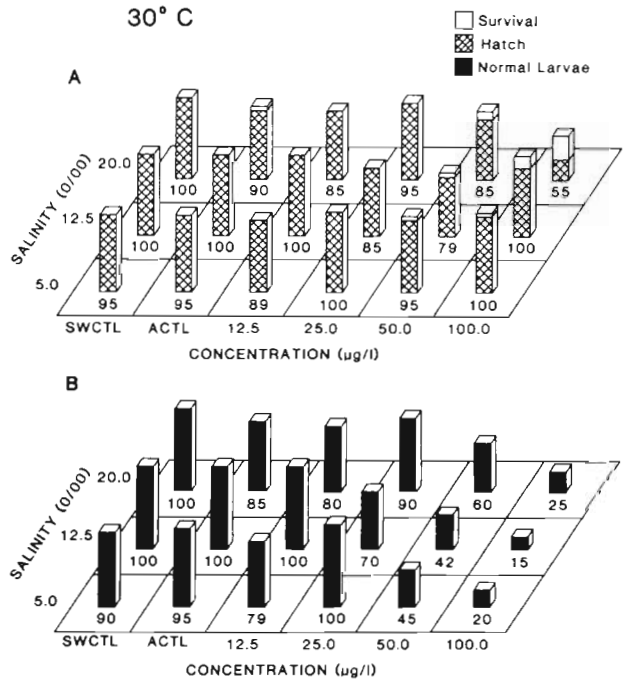

Fig. 3. *Menidia beryllina*. Results of blastula stage embryos exposed to terbufos at 30 °C. See Fig. 1 for further details

Table 4. Results of the multivariate regression analysis for the percentage of normal *Menidia beryllina* larvae from embryos exposed to terbufos; C = Concentration of terbufos (µg l^{-1}), S = Salinity (‰), T = Temperature (°C)

Source	df	SS	MS	F	p > F	r
Treatment	9	37.21	4.13	20.44	0.0001	0.84
Error	35	7.08	0.20			
Corrected total	44	44.29				
Interaction analysis						
C	1	25.18		121.50	0.0001	
S	1	0.37		1.81	0.1871	
T	1	7.22		35.69	0.0001	
C^2	1	2.77		13.69	0.0007	
S^2	1	0.22		1.06	0.3100	
T^2	1	0.63		3.13	0.0857	
C × S	1	0.68		3.35	0.0758	
C × T	1	0.11		0.55	0.4648	
S × T	1	0.04		0.20	0.6587	

Regression coefficients Parameter	Estimate
Intercept	−6.95999
C	−0.03765
S	0.10389
T	0.63124
C^2	0.00035
S^2	−0.00272
T^2	−0.01012
C × S	−0.00065
C × T	−0.00039
S × T	−0.00120

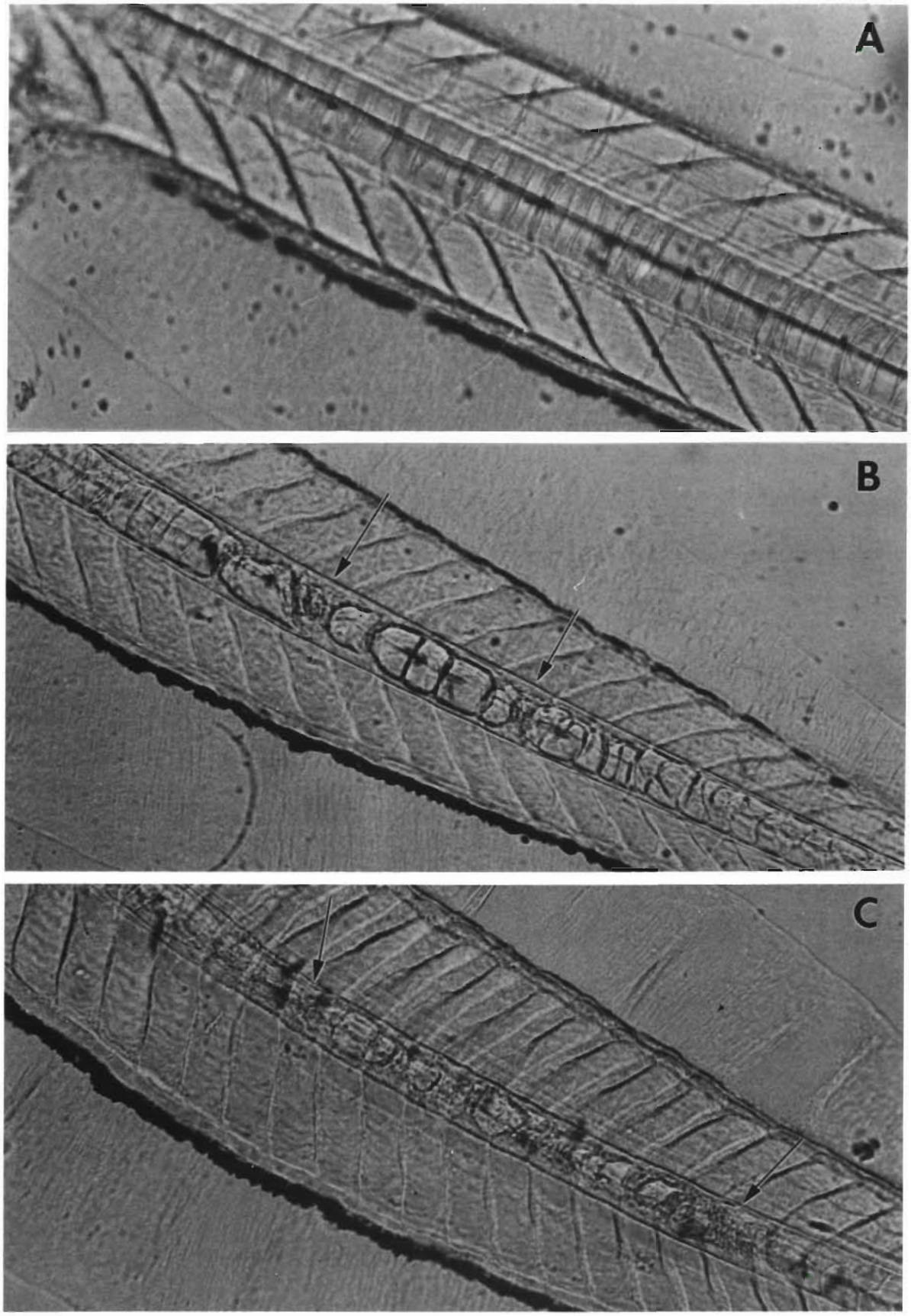

Fig. 4. *Menidia beryllina*. Vertebral columns of newly hatched embryos showing normal development and induced vertebral anomalies. (A) Embryo reared in clean seawater at 25 °C and 20 ‰ S; (B) embryos exposed to 25 µg terbufos l^{-1} at 25 °C and 12.5 ‰ S. Arrows indicate severly malformed centra; (C) embryo exposed to 50 µg terbufos l^{-1} at 25 °C and 5 ‰. Arrows indicate area of severely malformed vertebrae

tebrae from 2 terbufos exposed hatched larvae are shown in Fig. 4. A strong relationship existed between increased incidences of vertebral damage and increasing terbufos concentration. However, no trend in the severity of the vertebral malformations was observed in relation to increasing concentrations of terbufos.

Analysis of the percentage of normal larvae without vertebral anomalies (Figs. 1B, 2B and 3B), indicated significant differences between controls and terbufos exposed embryos ($p = 0.0001$), and also among the 3 temperatures utilized ($p = 0.0001$) – with the lower temperatures associated with increasing anomalies. Salinity did not exert a significant effect ($p = 0.175$), nor were there any significant interactions between the main factors of terbufos concentration, temperature orsalinity ($p \geq 0.921$). Post-hoc analysis revealed a significantly higher percentage of larvae without skeletal anomalies in controls vs embryos exposed to nominal concentrations of 25.0, 50.0 and 100.0 $\mu g\ l^{-1}$ terbufos. Some larvae with vertebral anomalies were observed in the 12.5 $\mu g\ l^{-1}$ treatment. Further analysis showed tests conducted at 20 °C resulted in a significant reduction in the percentage of normal larvae, and increased skeletal anomalies of 14 and 9 % when compared to tests run at 25 and 30 °C, respectively.

Results of the multivariate regression model for the percentage of normal larvae are shown in Table 4. The linear effects of concentration and temperature, and the quadratic effect of concentration are the only significant effects in the model. The response surface for the predicted percentage of normally developed larvae at each nominal concentration, for the 9 combinations of temperature and salinity is shown in Fig. 5. Temperature had the greatest predicted effect on the percentage of normal larvae. With increasing temperature an increase in the number of normal individuals was predicted. Terbufos had the opposite, predictable effect, an increase in concentration resulted in a decrease in the percentage of normally developed larvae. The predicted effect of salinity on the percentage of normal larvae was negligible, however, the plots indicate a trend toward decreasing percentages of normal larvae as salinity increases, at the higher terbufos concentrations.

DISCUSSION

Developmental success in many teleosts is affected by environmental factors such as temperature, salinity, pH and DO, acting singly or in concert on physiological processes. Temperature and salinity tolerance ranges

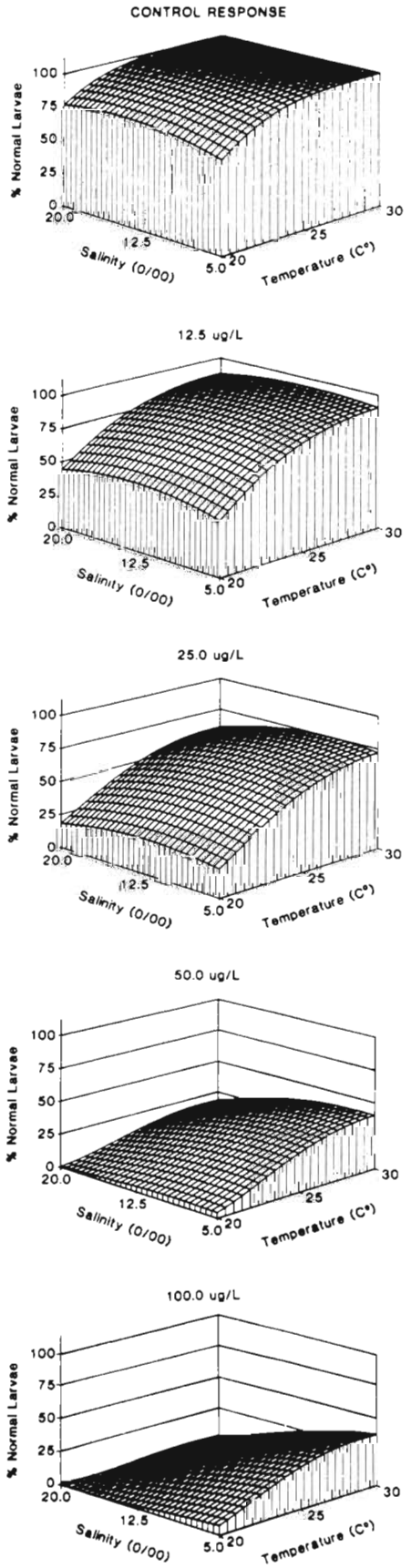

Fig. 5. *Menidia beryllina*. Response surface for predicted percentage of normally developed larvae at each nominal terbufos concentration for the 9 combinations of temperature and salinity

are typically narrow for marine organisms, but show a much wider range for species found in estuarine habitats. In reviews, Kinne (1963, 1964) described the interactive effect that temperature and salinity may have on one another. Temperature can alter the effects of salinity by increasing, decreasing, or shifting the range of salinity tolerance for a specific organism. Reciprocally, salinity can alter the temperature tolerance range.

Our results show that salinity had the least effect on normal development, survival and hatching of *Menidia beryllina* embryos. This is not surprising in light of the euryhaline nature of the species. *M. beryllina* resides along the Atlantic and Gulf coasts as well as in freshwater rivers, lakes and reservoirs of the USA. It is found in freshwater and at salinities of 75 ‰, but estuarine populations seem to prefer salinities of 19 ‰ or less (Middaugh et al. 1987). Using an estuarine population of *M. beryllina*, Middaugh et al. (1986) demonstrated no significant differences in hatching and larval survival for embryos maintained at 5, 15 and 30 ‰ and 25 °C.

Although an interaction between salinity and terbufos concentration did influence embryo survival at their respective highest values, the most prominent influence salinity displayed was on hatching success, resulting in a slight reduction in hatching for tests conducted at 20 ‰. Figs. 1A, 2A and 3A indicate a trend toward diminished hatching success with decreasing temperature, coupled with the combination of increasing salinity and terbufos concentration. Hubbs et al. (1971) reported similar temperature-salinity effects using embryos from a freshwater population of *Menidia beryllina* (*audens*) from Lake Texoma, Oklahoma. In that study, percentage embryo-larval survival at 20 °C ranged between 55 and 75 %, with combinations of low temperature and high salinity considerably diminishing hatching success. However, the Lake Texoma population also exhibited high survival at combinations of low temperature and low salinity. Conversely, control embryos in our study showed the lowest percentages of survival, hatching and normal development at the extreme of 20 °C and 5 ‰. This difference in response may be attributable to restriction of the lake population to a freshwater habitat with a resultant loss of euryplasticity, and, to latitudinal differences in temperature tolerance between the 2 groups, resulting in a greater resistance to lower temperatures in the lake population.

Although the estuarine populations are euryhaline, it appears that low temperature may decrease the salinity range for optimal survival and hatching in *Menidia beryllina*. Our data also indicate that, regardless of temperature, embryos maintained at higher salinities are more susceptible to the effects of terbufos, resulting in a lower percentage of normal larvae. Linden et al. (1979) exposed *Fundulus heteroclitus* to the water soluble fraction of No. 2 fuel oil and hypothesized that an embryo at non-optimal salinities may be unable to maintain its osmoregulatory capacity due to decreasing availability of metabolic energy at lower temperatures. This, coupled with the additional stress of a toxicant on metabolic maintenance, decreases the developmental success of an embryo. Indeed, terbufos-exposed embryos at 20 °C displayed decreased percentages of survival and hatching with increased percentages of skeletal anomalies. Weis et al. (1981) observed increased teratogenic effects of methylmercury to *F. heteroclitus* exposed at lower temperatures, and attributed the increased effects to prolongation of the developmental period thus allowing longer exposure to the toxicant during embryogenesis.

In the test conducted at 20 °C and 5 ‰ S, several seawater and acetone controls showed arrested development with subsequent degeneration of the craniofacial, carciovascular and skeletal systems resulting in death of the embryos before hatching. Research with other teleosts show that sub-optimal temperatures and salinities can influence biochemical and physiological processes leading to morphological malformations, alterations of developmental rate and lethality (Blaxter 1969, Rosenthal & Alderdice 1976, Laale & Lerner 1981).

Two additional acetone-control larvae in this test exhibited vertebral anomalies which were observed only in terbufos exposed individuals of the other tests conducted. Vertebral anomalies have been shown to occur frequently in both natural populations and laboratory reared fish (Gabriel 1944, Gill & Fisk 1966, Hickey et al. 1977). A study of temperature effects on vertebral development of chinook salmon *Oncorhynchus tshawytscha* embryos indicated an increase in the number of vertebral anomalies at temperatures both above and below optimum (Seymour 1959). Weis & Weis (1974) in a study of *Fundulus heteroclitus* embryos exposed to parathion and carbaryl, and Weis & Weis (1976) in a later study with *Menidia menidia* embryos exposed to DDT (dichlorodiphenyltrichloroethane), malathion and carbaryl, found that exposures to assorted insecticides of fundamentally different chemical structure, elicited similar developmental malformations. They speculated that a species may exhibit a tendency to develop specific anomalies in response to exposure to various chemical agents. Therefore, *M. beryllina* embryos may have a tendency to develop vertebral anomalies under sub-optimal environmental conditions which are augmented by exposure to terbufos. Moreover, the observed increase in vertebral anomalies associated with increasing terbufos concentration might be a condition of enhanced susceptibility

of biochemical, developmental and/or physiological processes to the synergistic action between sub-optimal environmental factors and terbufos exposure.

Organophosphorus compounds have been shown to produce skeletal deformities such as scoliosis and lordosis in a variety of fish species. Fathead minnows *Pimephales promelas* and brook trout *Salvelinus fontinalis* chronically exposed to diazinon developed varying degrees of scoliosis and lordosis ranging from small kinks to extreme spinal displacement (Allison & Hermanutz 1977). Diazinon was also reported to cause scoliosis and hemorrhaging around the dorsal fin in exposed Motsugo fish *Pseudorasbora parva* (Kanazawa 1975). Vertebral displacement with localized hemorrhaging resulting from exposure to organophosphorus compounds have also been documented by Eaton (1970) and McCann & Jasper (1972) for bluegill *Lepomis macrochirus*, Kumar & Ansari (1984) for zebrafish *Brachydanio rerio* and by Mount & Boyle (1969) for brown bullheads *Ictalurus nebulosus*. In most cases, the above mentioned displacements and hemorrhaging may be explained by the anticholinesterase action of the compounds inducing severe neuromuscular contractions which fracture the vertebrae. The vertebral anomalies expressed by the terbufos exposed embryos in our study ranged from slight distortions of the centra to multiple fusions of vertebrae along one or more sections of the vertebral column (Fig. 4). Although the mechanism is unknown, we feel these abnormalities are not mechanically produced by flexures, but caused by toxicant induced interference of developmental processes. One explanation for the occurrence of these anomalies may be in the improper formation of collagen by the fibroblast. Mayer et al. (1978) reported a significant decrease in the collagen content of channel catfish backbones after exposure to toxaphene. They suggested that competition for Vitamin C by enzymatic detoxification processes with organochlorine insecticides may have resulted in a net deficiency of Vitamin C necessary for collagen synthesis through the hydroxylation of proline. However Kozlovskaya & Mayer (1984) stated that exposure of crucian carp *Carassius carassius* to trichlorfon (organophosphate insecticide), unlike toxaphene, did not involve hydroxyproline. Rather, diminution of collagen formation with trichlorfon was probably a function of esterase inhibition.

In conclusion, our results showed that terbufos exposure reduced embryo survival and hatching and is teratogenic to developing *Menidia beryllina*. These effects may be exacerbated by sub-optimal environmental conditions resulting in a lower proportion of normally developed individuals. Further studies are underway to assess the effect of embryonic terbufos exposure on vertebral development during ossification and subsequent growth.

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