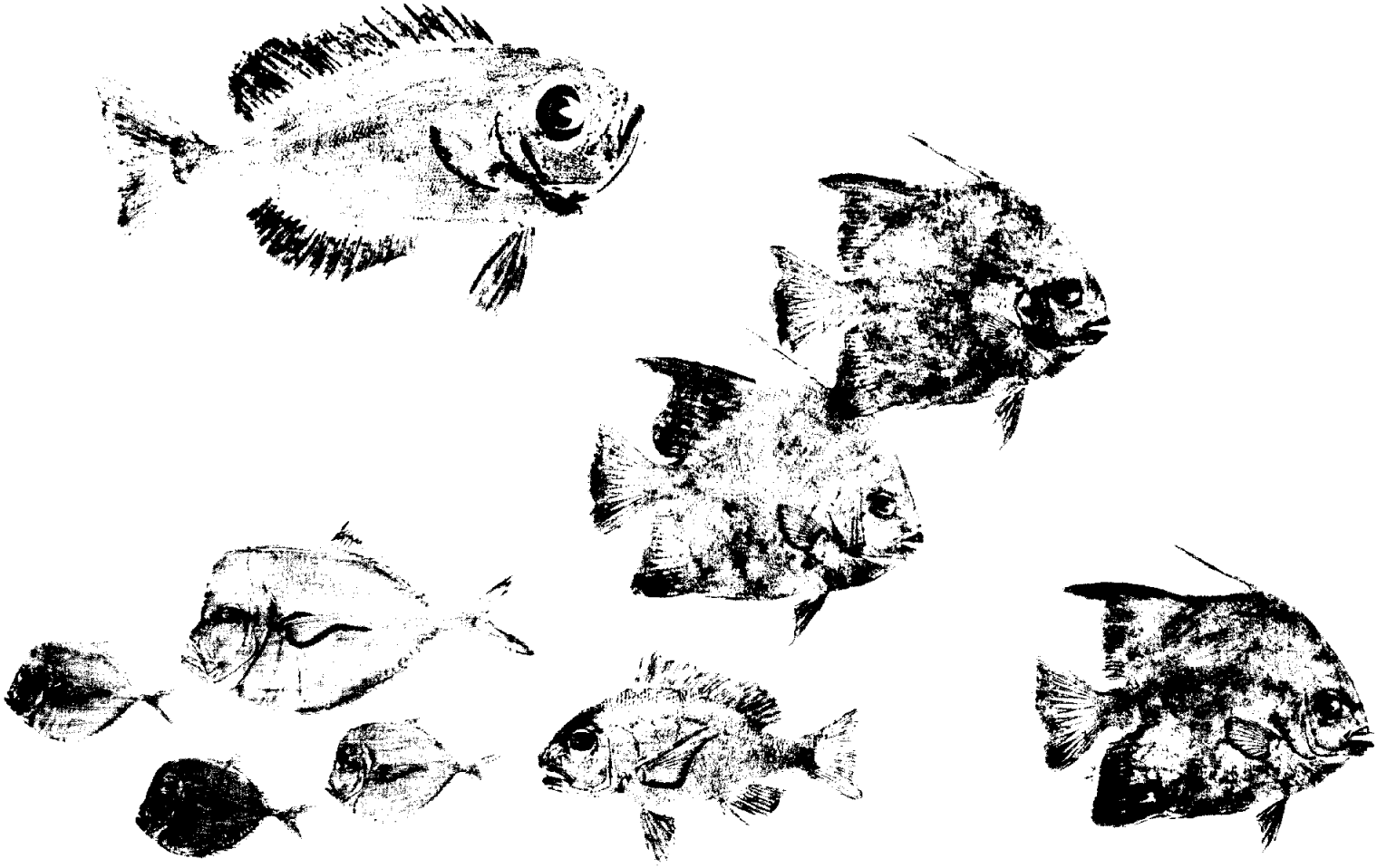

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SALINITY REQUIREMENTS FOR REPRODUCTION AND LARVAL DEVELOPMENT OF SEVERAL IMPORTANT FISHES IN TEXAS ESTUARIES

Final Report

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I REPRODUCTION IN SPOTTED SEATROUT AND ATLANTIC CROAKER

by

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II SALINITY TOLERANCE IN LARVAE OF SPOTTED SEATROUT, RED DRUM AND ATLANTIC CROAKER

by

G. Joan Holt and Mike Banks

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SUMMARY

Salinity extremes significantly impaired all phases of reproduction and larval development examined in spotted seatrout, Atlantic croaker and red drum, from the beginning of oocyte growth to several weeks post-hatching of the larvae. Several stages of the reproductive and early life history cycles of these sciaenid fishes were particularly susceptible to salinity stress. Salinity exerted a marked effect on reproductive endocrine function and oocyte growth in spotted seatrout during early stages of ovarian growth. A second period of sensitivity to salinity was observed at fertilization which was most evident in Atlantic croaker. Moreover the fertilized eggs of both species were buoyant over a narrow range of salinities. The fertilized eggs were susceptible to salinity extremes prior to the gastrula stage. A further period of decreased salinity tolerance was observed at the end of the yolk sac stage in 3-day old larvae. Finally, the growth rate was impaired during longer term exposure of fish larvae to salinity extremes.

There were differential effects of salinity on the reproduction and larval survival of the three sciaenid species. In general spotted seatrout were more tolerant to both low and high salinities than the other two species. Salinity ranges for successful reproduction and larval survival of spotted seatrout were approximately 20-45$^{o}/_{oo}$S and 10-40$^{o}/_{oo}$S, respectively, although reproductive output may be diminished in salinities different from the optimum (35$^{o}/_{oo}$S) and larval growth rate reduced at higher salinities (45$^{o}/_{oo}$S). Additionally, the larvae produced by adult spotted seatrout collected from Copano Bay (24$^{o}/_{oo}$S) were consistently less tolerant to high saline conditions (> 36$^{o}/_{oo}$S) than those obtained by spawning fish collected in Aransas Bay (32$^{o}/_{oo}$S). In croaker successful fertilization and hatching only occurred between 25 and 35$^{o}/_{oo}$S and larval development between 15 and 35$^{o}/_{oo}$S. Red drum larvae had a similar salinity tolerance (15-35$^{o}/_{oo}$S). Additional studies are required to refine these salinity tolerance estimations in light of possible population or acclimation differences, and the potential for salinity tolerance to be modified by temperature.

RECOMMENDATIONS FOR FUTURE WORK

1. Follow up on our findings that salinity tolerance of spotted seatrout larvae is influenced by parental stock suggesting that there are population differences in salinity tolerance.
   a. Investigate the effects of salinity on reproduction in spotted seatrout collected from different spawning locations and salinities.
   b. Further qualify the salinity tolerance of spotted seatrout larvae from different spawning salinities and/or different spawning populations.
2. Amass data needed to fine tune or adjust salinity limits defined in the present study.
   a. Investigate the interactive effects between temperature and salinity tolerance in larval fish.
   b. Determine the effects of salinity on frequency of spawning in spotted seatrout to estimate total annual fecundity.
   c. Describe and quantify changes in larval feeding and other behavior parameters potentially compromised by the outer salinities within the ranges tolerated by larval fish.
3. Begin an examination of endocrine mechanisms of reproductive impairment at high and low salinities, in particular, the involvement of prolactin and thyroid hormones in adults and eggs. Study the role of chloride cells in osmoregulation during larval development.
4. Investigate salinity effects on sexual maturation and gonadal growth in young of the year Atlantic croaker.
5. Examine salinity effects on reproduction in red drum.

INTRODUCTION

It has been widely documented that salinity is an important physical factor controlling the distribution of teleosts (Holliday, 1969). Estuaries being interfaces between fresh and saltwater are characterized by dynamic saline conditions where excessive precipitation or evaporation may exacerbate fluctuations to extreme hypo- or hypersaline status. Thus, competent osmoregulation through a broad range of salinities is an important, if not limiting, criterion for teleosts living in estuarine regions. Successful reproduction and larval recruitment are particularly sensitive to altered environmental conditions including salinity changes (Billard *et al.*, 1981; Gerking, 1980). Therefore major changes in freshwater inflow through upstream allocation of water, could have long-term consequences on fish populations dynamics. Although moderate increases in salinity may not be acutely lethal to estuarine fishes, resultant increases in energy requirements for acclimation leave fewer energy reserves for growth and reproduction.

Teleost reproduction is a complex process involving considerable physiological coordination which is largely controlled by endocrine glands. Peptide and steroid hormones secreted by these glands control the timing of reproduction (Peter and Crim, 1979), the mobilization of energy reserves for gonadal development (Ng and Idler, 1983; Billard *et al.*, 1982), final egg maturation (Goetz, 1983) and breeding behavior (Demski and Hornby, 1982). Adverse environmental stimuli can alter fish reproduction by impairing reproductive endocrine function (Billard *et al.*, 1981). Corticosteroid hormone secretion is also altered during exposure to adverse stimuli (Thomas *et al.*, 1980) and may secondarily affect reproductive function.

Immediately prior to ovulation and spawning the fully grown eggs undergo a process called final oocyte maturation (FOM, Goetz, 1983). During FOM the eggs absorb water and swell to several times their original size. Adequate hydration of eggs appears to be necessary for successful fertilization and hatching. It is likely that this process would be particularly sensitive to alterations in the ambient salinity. However, interactions between the process of final oocyte maturation and ambient salinity remain to be explored in most marine fishes, including spotted seatrout and Atlantic croaker.

For fish embryos and larvae, osmoregulation is particularly problematic. The organ system resources and strategies (renal-branchial-gut) employed by juvenile and adult fishes are in the process of differentiation in larvae and are thus not immediately available to larvae. Not surprisingly, salinity tolerance has been found to be most narrow in many species during early ontogeny (Kinne, 1964). Holliday (1969) postulated that the ability for larvae to survive changes in salinity depended upon either or both of two factors. First, the ability of the body fluids to function at least for a short time in an abnormal range of internal osmotic and ionic concentration and, second, the ability of the larvae to regulate the body fluids in order to restore the osmotic pressure to near normal.

Shen and Leatherland (1978) suggested the possibility of osmoregulation at the cellular rather than organismal level in developing fish larvae. Chloride cells, found in the integument of very young embryos may represent an intermediary, cellular means of achieving osmoregulation during early development (Alderdice, 1988). Thus, active osmoregulatory mechanisms may be present well before hatching, although their location and morphology may deviate from this in the later life stages (Riis-Vestergaard, 1987).

The vast majority of Gulf fisheries species (over 97.5%) are estuarine-dependent although the seasonal aspects of their life cycles are quite different (Texas Dept. of Water Resources, 1979). Fish species spawn at different times and with different migratory patterns, often utilizing the estuarine nursery habitats in different seasons and during different stages of their life cycles. Pearson (1929) noted that spotted seatrout (*Cynoscion nebulosus*) spawn largely in primary and secondary bays. Numerous reports have confirmed that spawning occurs at dusk during summer months, commencing in late March and early April and continuing until late September (Tabb, 1961, 1966; Holt *et al.*, 1985; Brown-Peterson *et al.*, 1988; Peebles and Tolley, 1988). Red drum (*Sciaenops ocellatus*) spawn in coastal nearshore waters, within reach of estuarine systems, during late summer and early fall. Their planktonic larvae are carried inshore to estuarine nursery areas by the tides and currents (Pearson 1929; Mansueti, 1960; Loman, 1978; Perret *et al.*, 1980; Holt *et al.*, in press). Atlantic croaker (*Micropogonius undulatus*) migrate from estuarine waters in the fall at a time of decreasing photoperiod and dropping temperatures to spawn in warmer (18 to 25° C) offshore Continental shelf waters (Fahay, 1975), and are recruited into estuarine nursery areas only during post-flexion stages (Lewis and Judy, 1983).

Considering the important modifying effects of changes in freshwater inflow to the saline conditions of estuarine systems, establishment of the limits within which healthy populations can be maintained is imperative. The overall objective of this study was to determine the range of salinities in which the reproduction and larval survival of valuable estuarine fish would not be significantly impaired as a result of alterations in fresh water inflow into Texas bays and estuaries. The effects of different salinities on gonadal development, ovulation, hatching success and survival and growth of larval fish were investigated. In

addition, sublethal effects of salinity stress on reproductive endocrine function, as well as the ontogeny of physiological adaptation of eggs and larvae were examined.

I. REPRODUCTION IN SPOTTED SEATROUT AND ATLANTIC CROAKER

MATERIALS & METHODS

Adult spotted seatrout (800-1600 g BW) (oocytes ≤ 140 μm) were collected by gill net in the vicinity of Port Aransas, Texas between January and March prior to the period of ovarian recrudescence. Adult Atlantic croaker in late stages of ovarian recrudescence were captured via gill net and hook-and-line near Port Aransas during September and October. Fish were acclimated to laboratory conditions in 4000 L circular tanks with external biofilters (salinity $30^{o}/_{oo}$S) for three weeks and fed shrimp twice daily at the rate of 3-5% of their body weight per day.

TREATMENTS

A. Effects of salinity on reproductive endocrine function and ovarian growth.

At the end of the acclimation period the fish were divided equally among eight eight-foot diameter (4000 L) tanks. The salinity in each tank was then adjusted by adding a synthetic sea salt mixture (Fritz Chemical Co., Dallas) or dechlorinated tap water.

The salinities in the seatrout experiments were changed at the rate of $5^{o}/_{oo}$ per day until the final salinities were reached, $10^{o}/_{oo}$S, $20^{o}/_{oo}$S, $35^{o}/_{oo}$S and $45^{o}/_{oo}$S. Each salinity treatment was replicated. Water temperature was maintained at 25 ± 1° C and the

photoperiod regime was set at 13L:11D. Spotted seatrout were sampled before (control) and after 30 and 60 days of exposure to the test salinities.

The salinities in the croaker experiments were adjusted over a 10 day period ($5^{o}/_{oo}$S, $15^{o}/_{oo}$S, $25^{o}/_{oo}$S, $35^{o}/_{oo}$S, $45^{o}/_{oo}$S). Photoperiod and temperature were adjusted to mimic Fall conditions with a photoperiod of 12L:12D and a temperature range of 22-24°C. Croaker were sampled after 22-25 days exposure to the test salinities. The entire experiment was repeated during a subsequent reproductive season.

Fish were rapidly captured between 8:00 and 10:00 am on the last day of the experiment and blood was collected within five minutes from the caudal vein. Blood samples were centrifuged and the plasma stored at -80° C until analyzed for gonadal steroids. Body weight (BW) and gonad weight (GW) were measured to the nearest 0.1 gram. Gonadosomatic index (GSI) values were calculated by the following formula:

$$GSI = (GW/BW) \times 100$$

Gonads were preserved in Bouin's fixative or 10% phosphate buffered formalin and later a small portion in the middle of one lobe of the gonad was embedded in paraplast plus tissue embedding medium (Monoject Scientific, St. Louis). Embedded tissues were sectioned at 6-8 μm and stained with hematoxylin and eosin. Five-ten sections from each ovary were randomly selected to examine developmental stages of oocytes. For the seatrout, in each section, the number of oocytes in each developmental stage in the visual field of 100X magnification under light microscope (areas: 2.54 μm^2) were tallied. For croaker 100 randomly chosen oocytes were examined to determine developmental stage. Plasma estradiol (E2) and testosterone (T) levels were measured by radioimmunoassay

(RIA) procedures validated for hormone measurement in spotted seatrout and Atlantic croaker plasma as described previously (Singh *et al.*, 1988).

B. Spawning and hatching success.

After acclimation to laboratory conditions the salinities in the experimental tanks were adjusted over a ten day period to the final test salinities. Fish were exposed to these salinities for 1-3 months and induced to spawn at the test salinities by LHRHa injection (Thomas and Boyd, 1988).

Spotted seatrout were exposed to $10^{o}/_{oo}$S, $15^{o}/_{oo}$S, $25^{o}/_{oo}$S, $35^{o}/_{oo}$S or $50^{o}/_{oo}$S for up to four months. Photoperiod and water temperature were adjusted to mimic local conditions beginning with a photoperiod of 11L:13D and 18° C in April and progressing to 15L:9D and 28° in August and then held constant until the conclusion of the experiments. The $10^{o}/_{oo}$S, $15^{o}/_{oo}$S, and $50^{o}/_{oo}$S exposures were repeated in July with females at a midpoint in ovarian recrudescence (oocytes $\simeq$ 250 μm). Handling and injection of fish to induce spawning had resulted in the death of all the females held at these salinities in the first experiment.

Atlantic croaker were exposed to $5^{o}/_{oo}$S, $15^{o}/_{oo}$S, $25^{o}/_{oo}$S, $35^{o}/_{oo}$S or $45^{o}/_{oo}$S for 1-3 months. The entire experiment was repeated during a subsequent reproductive season. Photoperiod and temperature regimes were adjusted during the exposure period to mimic seasonal changes during the Fall and early Winter.

Fecundity, percent fertilization, percent hatch of fertilized eggs, fertilized egg diameters, percent abnormal larvae and larval survival up to 4 days post hatch were investigated. Neutral buoyancy for croaker eggs fertilized in $15^{o}/_{oo}$S, $25^{o}/_{oo}$S and $35^{o}/_{oo}$S salinity by placing 10 eggs in various salinities ($16^{o}/_{oo}$-$34^{o}/_{oo}$S) and noting the salinity at which approximately

50% of the eggs sank. Fertilization, hatch and survival were determined from a random sample of 10-40 eggs from each spawn placed in 50 milliliter glass culture dishes. Ten replicates per spawn were used for egg and larvae incubation at the spawning salinity (=test salinities) while 3 replicates were set up for each of the various transfer/hatching salinities ($5^{o}/_{oo}$-$50^{o}/_{oo}$) into which the fertilized eggs were transferred 1-3 hours after fertilization. Statistical significance at the P = .05 level was determined with either a one-way ANOVA followed by Duncan's multiple range test or a Kruskal-Wallis followed by Mann-Whitney tests.

RESULTS

A. Reproductive endocrine function and ovarian growth.

1. Spotted Seatrout

Individuals in the $5^{o}/_{oo}$S tanks died within 17 to 25 days after the beginning of the experiment. Between Days 38 and 43 the fish in 10 ppt salinity group died of a *Vibrio* infection. Therefore, all the data from the $5^{o}/_{oo}$S group and part of the data from the $10^{o}/_{oo}$S groups were not included in the analysis.

Males

No significant differences in the male GSI values among the treatment groups were observed after either thirty or sixty days exposure to the various salinities (Fig. 1a). Mean plasma testosterone levels of fish held at $45^{o}/_{oo}$S and $20^{o}/_{oo}$S were significantly higher than those held at $35^{o}/_{oo}$S and $10^{o}/_{oo}$S after thirty days exposure, but by sixty days mean values for the $20^{o}/_{oo}$S, $25^{o}/_{oo}$S and $45^{o}/_{oo}$S groups were not significantly different (Fig. 1b). Before the experiment the testes were in an early stage of spermatogenesis and contained

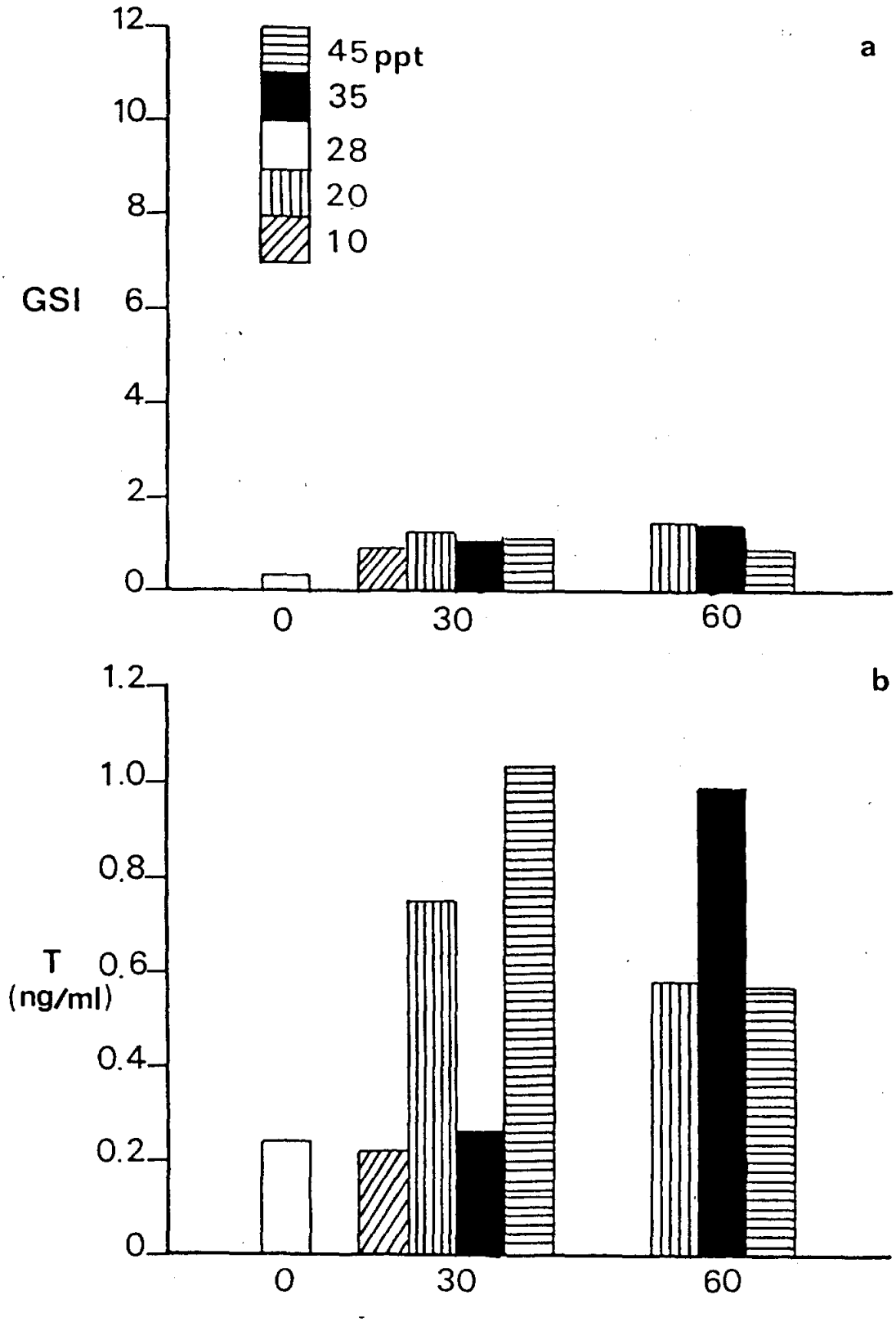


Figure 1. (a) GSI values and (b) serum testosterone (T) levels (ng/ml) of male spotted seatrout at the beginning (Day-0), middle (Day-30) and the end (Day-60) of exposure to various test salinities (10 ppt, 20 ppt, 35 ppt and 45 ppt). Initial salinity 28 ppt.

primarily spermatogonia and spermatocytes, although a few spermatids were also present (Fig. 2a). By Days 30 and 60 the testes had advanced to the spermatogenesis stages in all treatment groups and spermatozoa could be observed in the lumen and in the sperm duct (Figs. 2 b,c).

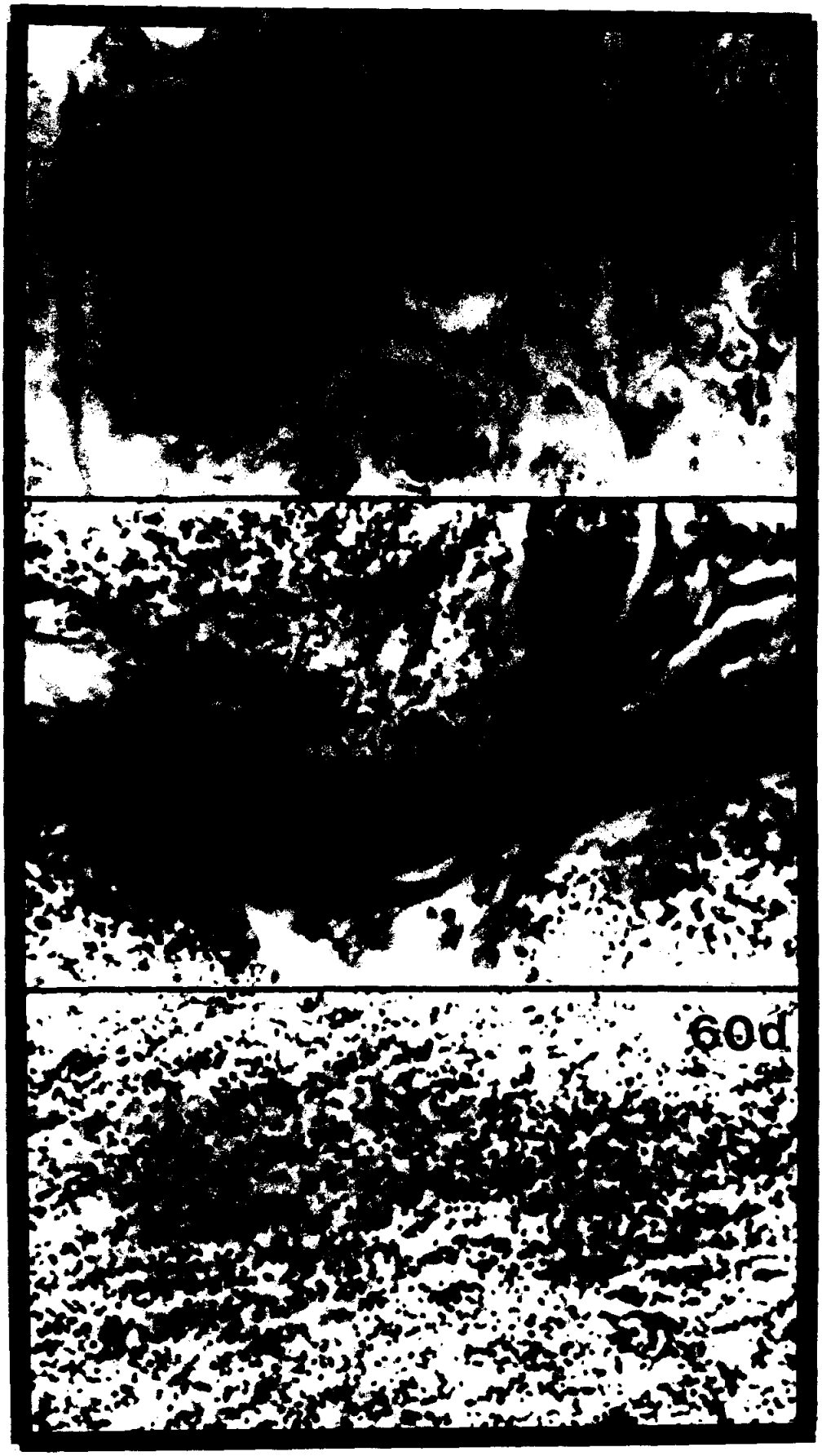
The GSI values, plasma testosterone levels and histological results indicate that spermatogenesis and male reproductive endocrine function is not significantly altered in male spotted seatrout by the salinity range (20-45°/$_{00}$S) tested in this experiment.

Females

The mean GSI of females held at 20°/$_{00}$S, 35°/$_{00}$S and 45°/$_{00}$S increased during the first 30 days of the experiment, although the increase was only significant in the 35°/$_{00}$S group which had the highest values ($P<0.04$, Fig. 3a). There was a further increase in GSI from Days 30 to 60 which was significant in the 35°/$_{00}$S ($P<0.001$) and 45°/$_{00}$ ($P<0.02$) groups (Fig. 3a). Highest mean GSI values were recorded in the 35°/$_{00}$S group, followed by the 45°/$_{00}$S treatment group. Mean GSI values of fish held at 20°/$_{00}$S for 60 days were approximately half of those held at 35°/$_{00}$S (Fig. 3a).

In general salinity-induced changes in circulating levels of gonadal steroids paralleled those observed with ovarian growth (GSI, Figs. 3 a,b,c). Plasma estradiol levels were increased after 30 days in females exposed to 20°/$_{00}$S, 35°/$_{00}$S and 45°/$_{00}$S seawater. Testosterone was also elevated in the 35°/$_{00}$S and 45°/$_{00}$S groups. As observed with the GSI, maximum circulating levels of both estradiol and testosterone were recorded in the fish exposed to 35°/$_{00}$S seawater. Plasma levels of estradiol and testosterone did not change significantly between Day 30 and Day 60 in fish exposed to the two highest salinities (Figs. 3 b,c). There was, however, a significant decline in estradiol concentrations in the 20°/$_{00}$S

Figure 2. Photomicrographs of testes of spotted seatrout at the beginning (0d), middle (30d) and the end (60d) of the experiment. Salinity was 35 ppt.



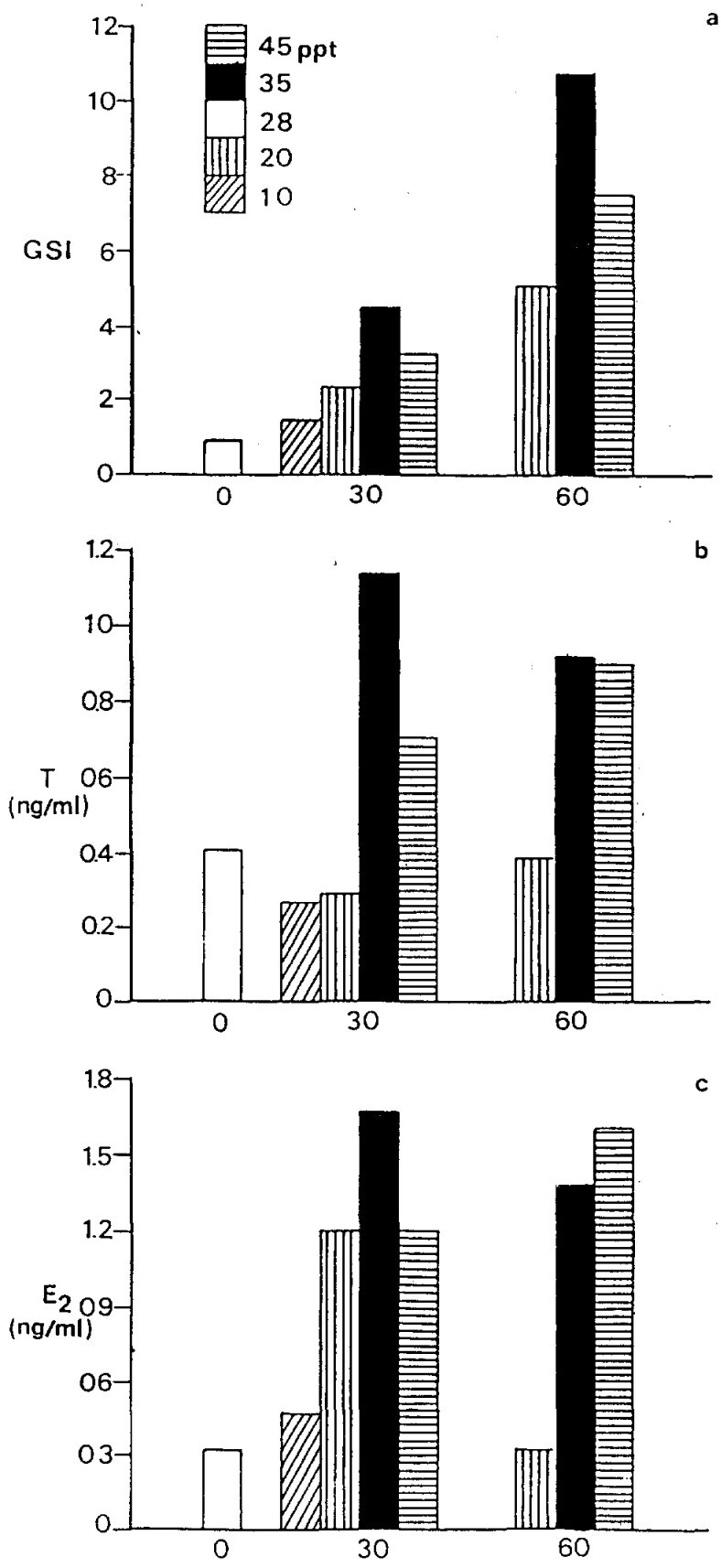


Figure 3. (a) GSI values (b) serum testosterone (T) levels (ng/ml) and (c) serum estradiol (E2) levels (ng/ml) of female spotted seatrout at the beginning (Day-0), middle (Day-30) and the end (Day-60) of the experiment. Salinities tested including 10 ppt, 20 ppt, 35 ppt and 45 ppt.

group during this period. At the beginning of the experiment, all females were at the same stage of gonadal recrudescence and contained only perinucleolus stage oocytes (Fig. 5). By Day 30, oocytes in the yolk vesicle stage, the primary yolk globule stage and atretic oocytes were also observed (Fig. 4). Fish held in $35^{o}/_{oo}$S sea water had a significantly higher percentage (28.2%) of oocytes at the primary yolk globule stage (Fig. 6). Fish held at $45^{o}/_{oo}$ and $20^{o}/_{oo}$S had 14.5% and 13.3% primary yolk globule stage oocytes at this time, significantly lower than the $35^{o}/_{oo}$S groups. Only 3.6% of the oocytes in the $10^{o}/_{oo}$S group were at the primary yolk globule stage, significantly lower than all the other groups ($P<0.05$). In contrast, fish held at $10^{o}/_{oo}$S had a significantly higher percentage of atretic oocytes (7.6%) than in the other three groups (0.8 - 1.53%). By the end of the experiment 44.1% of the oocytes in fish held at $35^{o}/_{oo}$S had reached the primary yolk globule stage, versus 34% in fish held at $5^{o}/_{oo}$S. The fish held at $20^{o}/_{oo}$S had only 28.1% of their oocytes in the primary yolk globule stage, significantly lower than the $35^{o}/_{oo}$S group (Fig. 6). The percentages of atretic oocytes in fish held at $20^{o}/_{oo}$S, $35^{o}/_{oo}$S and $45^{o}/_{oo}$S were 6.2%, 4.7% and 3.4% respectively.

The GSI values, plasma estradiol and testosterone concentrations and histological data indicate that reproductive endocrine function and ovarian growth in female spotted seatrout is significantly altered by salinities in this range (20-$45^{o}/_{oo}$S). Optimum conditions for ovarian recrudescence occurred at $35^{o}/_{oo}$S. Exposure to $20^{o}/_{oo}$S caused a greater suppression of ovarian growth than exposure to $45^{o}/_{oo}$S, which suggests that exposure to low salinities may cause greater disruption of reproductive processes in spotted seatrout than exposure to a higher salinity. Interestingly, exposure to $5^{o}/_{oo}$S and $10^{o}/_{oo}$S resulted in significant mortality of the reproductive females. These results suggest that females at

Figure 4. Photomicrographs of ovaries of spotted seatrout after 30 days exposure to 10‰S (10), 20‰S (20), 35‰S (35) and 45‰S (45) sea water. P - primary oocytes, PE - perinucleolus stage oocytes, YV - yolk vesicle stage oocytes, YG - yolk globule stage oocytes, AT - atretic oocytes.

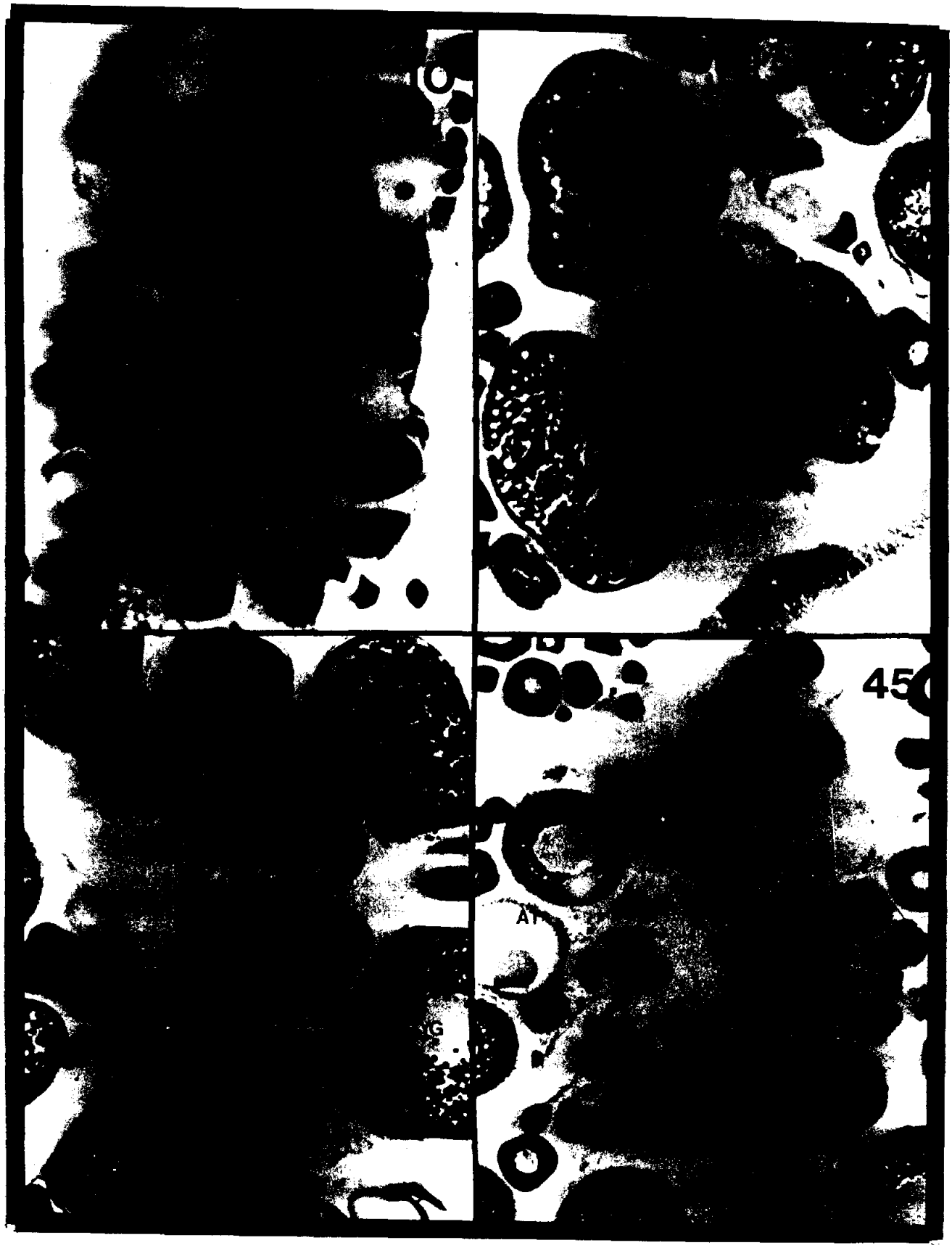
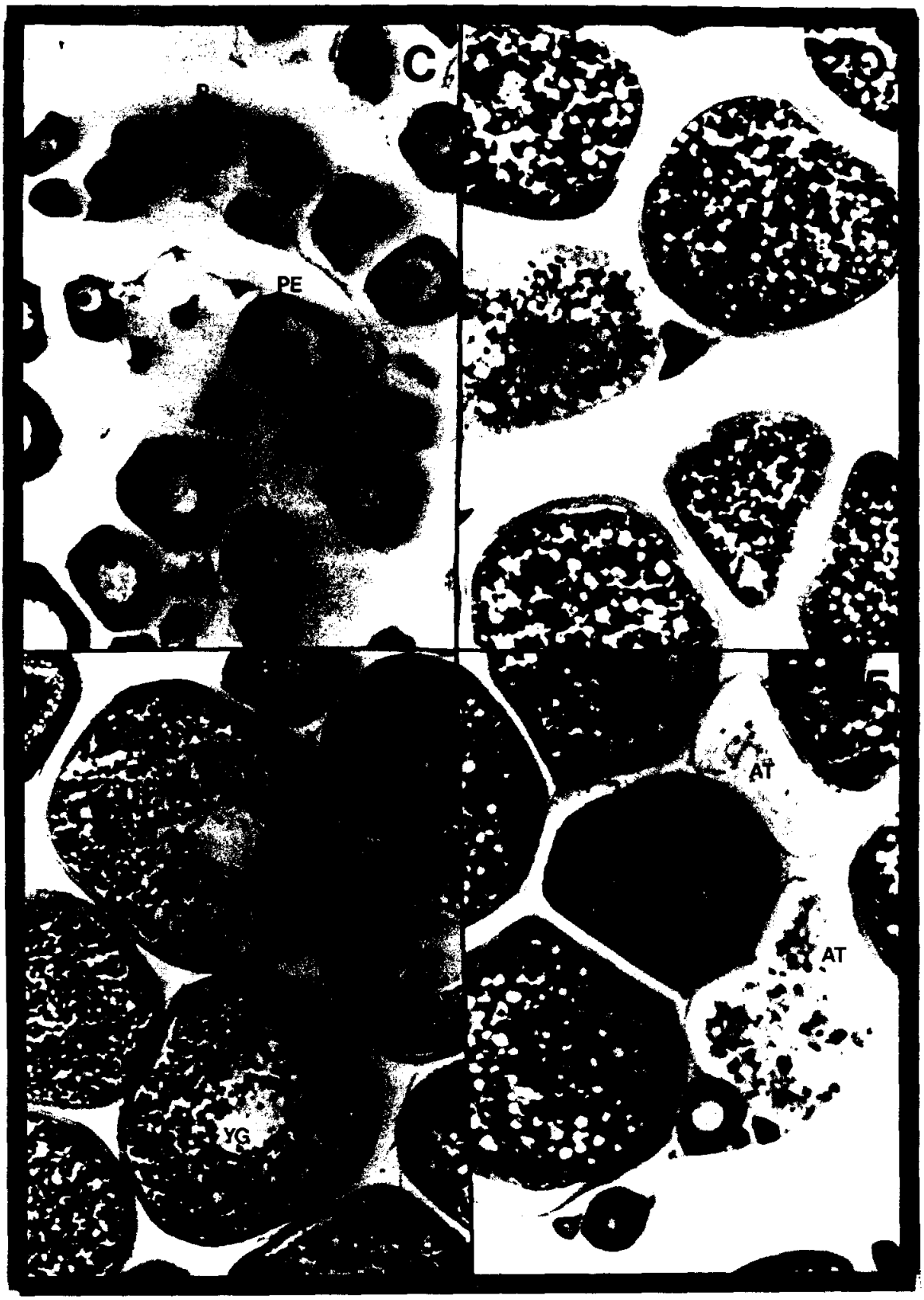


Figure 5. Photomicrographs of ovaries of spotted seatrout at the beginning (C) and after 60 days of exposure to 20‰S (20), 35‰S (35), and 45‰S (45) sea water. For explanation of symbols refer to Fig. 4 legend.



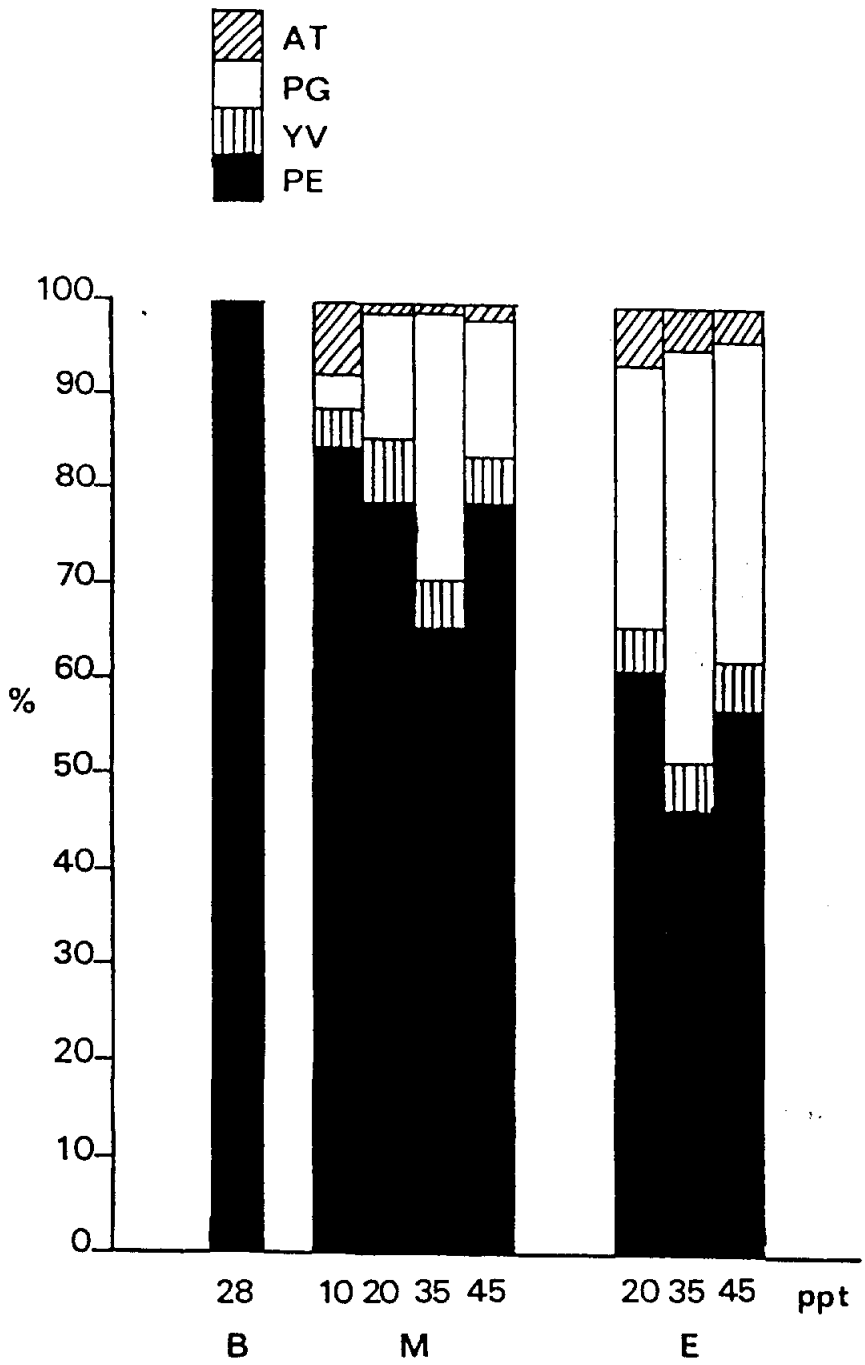


Figure 6. Percentage composition of various ovarian recrudescent stages of spotted seatrout at the beginning (B) (Day-0), middle (M) (Day-30) and the end (E) (Day-60) of exposure to various test salinities (10 ppt, 20 ppt, 35 ppt and 45 ppt). PE - perinucleolus oocyte; YV - yolk vesicle oocyte; PG - primary yolk globule oocyte; AT - atretic oocyte.

this life history stage may be susceptible to hypoosmotic stress. It is likely that the stress associated with holding these fish in captivity had an additive effect on the salinity-induced mortality. Further evidence that reproductive females held in suboptimal salinities are stressed and are particularly susceptible to additional stressors comes from the hormone-induction experiments described in the next section. Injection of the fully mature females with LHRH caused 100% mortality in the $10^{o}/_{oo}$S, $15^{o}/_{oo}$S and $50^{o}/_{oo}$S groups in the first experiment, but no deaths in the $25^{o}/_{oo}$S and $35^{o}/_{oo}$S groups. In marked contrast, none of the male fish died following these treatments. Thus it is concluded the female spotted seatrout are particularly sensitive to altered salinity during the reproductive season and have a diminished tolerance to subsequent stressors, whereas male reproduction appears to be largely unaffected.

2. Atlantic Croaker

Adult croaker survived and fed well in all test salinities. The ovaries of the experimental animals were at an advanced stage of recrudescence at the beginning of the experiment in 1987 and showed no further changes after 25 days exposure to any of the test salinities (Table 1). Similarly no salinity-dependent difference in plasma and testosterone concentrations were observed. However the gonadosomatic index and was significantly lower in the $45^{o}/_{oo}$ group than in the $35^{o}/_{oo}$S (control) group after 22 days of exposure in the 1988 experiments (Table 2). Circulating estradiol levels were also lowest in the $45^{o}/_{oo}$S group. Morphometric analysis of the different oocyte stages in histological sections of the ovaries revealed that in the 45 $^{o}/_{oo}$ group there was a reduction in the percentage of mature (yolk globule) oocytes compared to most of the other groups (49% vs 62%) and an increase in the percentage of atretic oocytes (8.2% vs 4%, Table 3). Male

TABLE 1. GSI, estradiol and testosterone levels of female croaker after 25 days of exposure to test salinities in 1987 (x ± 1 S.E.M.). Means with the same letter are not significantly different.

Test Salinity (‰)	Testosterone (ng/ml)	Estradiol (ng/ml)	GSI
5	2.25 ± 0.24^{A}	1.78 ± 0.16^{A}	18.6 ± 1.0^{A}
15	1.50 ± 0.08^{A}	1.56 ± 0.12^{A}	15.1 ± 0.5^{A}
25	1.69 ± 0.26^{A}	1.87 ± 0.3^{A}	17.3 ± 1.1^{A}
35	1.53 ± 0.21^{A}	1.36 ± 0.19^{A}	16.1 ± 0.8^{A}
45	2.27 ± 0.25^{A}	2.79 ± 0.29^{B}	17.7 ± 0.8^{A}

TABLE 2. GSI, estradiol and testosterone levels of female croaker after 22 days of exposure to test salinities in 1988 (x ± 1 S.E.M.). Means with the same letter are not significantly different.

Test Salinity (‰)	Testosterone (ng/ml)	Estradiol (ng/ml)	GSI
5	1.06 ± 0.18^{A}	$1.140 \pm 0.12^{A,C}$	17.1 ± 1.6^{B}
15	2.28 ± 0.49^{B}	2.551 ± 0.47^{D}	$19.9 \pm 3.5^{A,B}$
25	$1.59 \pm 0.31^{A,B}$	1.403 ± 0.21^{C}	11.2 ± 1.7^{A}
35	1.07 ± 0.17^{A}	0.861 ± 0.06^{B}	18.1 ± 1.0
45	$1.69 \pm 0.21^{A,B}$	$0.595 \pm 0.17^{A,B}$	8.6 ± 1.1^{A}

TABLE 3. Percentage composition of different oocyte development stages in ovaries of Atlantic croaker exposed to various salinities for 22 days. Means with the same letter are not significantly different.

Test Salinity (‰)	PERCENT				
	perinuclear	yolk vesicle	early yolk globule	yolk globule	atretic
5	25.4 ± 3.8^{A}	3.6 ± 1.1^{A}	5.6 ± 0.8^{A}	61.3 ± 3.9^{A,B}	4.1 ± 1.1^{A}
15	22.6 ± 2.0^{A,B}	6.4 ± 0.5^{A}	3.4 ± 0.6^{A,B}	62.4 ± 2.3^{A}	5.3 ± 0.8^{A}
25	37.1 ± 5.0^{B}	4.6 ± 0.7^{A}	5.5 ± 1.5^{A}	50.4 ± 5.6^{A,B}	2.4 ± 0.4^{A}
35	25.1 ± 2.7^{A,B}	5.4 ± 1.1^{A}	2.6 ± 0.9^{A,B}	62.9 ± 2.2^{A}	4.0 ± 0.9^{A}
45	37.0 ± 6.8^{B}	3.5 ± 0.5^{A}	2.0 ± 0.8^{B}	49.3 ± 5.2^{B}	8.3 ± 2.0^{B}

reproductive physiology was not investigated. However it was noted that at the time of LHRHa injection after 2 to 3 months exposure to $45^{o}/_{oo}$ salinity, sperm did not flow freely in response to light abdominal exposure as was observed with the other salinities.

It is concluded from these results that the final stages of oocyte growth in Atlantic croaker are relatively resistant to salinity extremes. There was some evidence in one experiment, however, for a decrease in fecundity and reproductive endocrine function and an increase in oocyte atresia in croaker exposed to $45^{o}/_{oo}$ salinity seawater.

B. Spawning and hatching success.

1. Spotted Seatrout

The spawning studies showed that females transferred to different salinities at a midpoint in ovarian recrudescence were able to complete oocyte development within 6-8 weeks and produce viable eggs. This suggests that later stages of egg development can occur at a wide range of salinities, although the rate of development may be slower at suboptimal conditions.

The spawning data revealed no marked differences in the total number of eggs spawned (Table 4). However there was a significant reduction in fecundity/Kg body weight in the $50^{o}/_{oo}$S group (296,000 vs 626,250 or greater in the other groups). The fertilization success was above 70% in all salinities except the $15^{o}/_{oo}$ and $50^{o}/_{oo}$S groups. The low fertilization success in the $15^{o}/_{oo}$ salinity group may be due to only one male being present in this tank versus two males in the other tanks. Percent hatch was 70% or above in all tanks except the $50^{o}/_{oo}$S group. Reliable data could not be obtained for 1 day survival. However, the data do confirm the poor survival in the $50^{o}/_{oo}$S group.

TABLE 4. Summary of spawning data for spotted seatrout held at various salinities for 60 days. Means with the same letter are not significantly different.

Salinity	# of Replicate Spawns	# Male/ # Female	Fertilized Egg Diameter (μm)[1]	# Eggs Kg Female	Percent Fertilization[1]	Percent of Fertilized Eggs Hatched[1]	Percent 1 Day Survival[1]
10	1	2/1	847.5 ± 6.0^{A}	1,141,167^{A}	73.0[1] ± 4.4^{A}	83.2 ± 3.6^{A}	62.7 ± 6.3^{2A}
15	1	1/1	833.1 ± 4.5^{A}	626,250^{A}	15.8 ± 2.0^{B}	62.5 ± 11.6^{A,B}	20.0 ± 10.5^{B}
25	1	2/3	734.4 ± 5.4^{B}	576,150^{A}	71.2 ± 8.6^{B}	87.8 ± 2.3^{A}	52.2 ± 5.9^{A}
35	2	3/3	654.4 ± 1.4^{B}	677,824 ± 186,648^{2A}	80.8 ± 4.8^{B}	80.6 ± 4.7^{A}	50.9 ± 5.3^{A}
50	1	2/3	636.3 ± 3.4^{B}	296,366^{A}	42.7 ± 4.4^{C}	33.1 ± 6.5^{B}	15.0 ± 10.7^{B}

[1] $\bar{x}$ ± S.E.M.

[2] Temperature significantly lower (24.8° vs 27° C)

Eggs spawned at the various salinities and subsequently transferred to a range of salinities (5-50°/$_{00}$S) in general showed high rates of hatching and 1 day survival (Tables 5 & 6). Hatching was only impaired at the highest (50°/$_{00}$S) and lowest (5°/$_{00}$S) salinities. Interestingly, failure to hatch at 5°/$_{00}$S was only observed for eggs spawned at high salinities. The 1 day survival data showed similar trends, with no survival only at the salinity extremes (Table 6).

It is concluded from these results that spotted seatrout are able to spawn successfully in a wide range of salinities and produce large numbers of fertilized eggs with high rates of hatching and 1 day survival. Thus the spawning and short term survival does not appear to be particularly sensitive to ambient salinity.

2. Atlantic Croaker

LHRHa injections resulted in eggs being released in all salinities with fertilization taking place in all except the 5°/$_{00}$ salinity and highest percent fertilization at 25°/$_{00}$ and 35°/$_{00}$ salinities (Table 7). Similarly, fecundity, as measured by the number of eggs collected per kilogram of female, appeared to be highest at 25°/$_{00}$. Neutral buoyancy for eggs spawned at 15°/$_{00}$, 25°/$_{00}$ and 35°/$_{00}$ was at 25°/$_{00}$, 27°/$_{00}$ and 35°/$_{00}$ respectively. These tests indicate that a majority of the eggs fertilized at these salinities in our study would sink in salinities less than 25°/$_{00}$. This result indicates that complete recovery of eggs from the spawning tanks at 5°/$_{00}$-25°/$_{00}$ may be difficult and could result in an underestimate of fecundity at these salinities. These buoyancy determinations are paralleled by the fertilized egg diameters which tended to be inversely related to spawning salinity (Table 7).

Hatching occurred in all spawning salinities with fertilized eggs except 45°/$_{00}$, where all embryos died before hatching. Although the percent of fertilized eggs eventually hatching

TABLE 5. Summary of spotted seatrout hatching success for eggs spawned at different salinities and transferred to various salinities of sea water.

Spawning Salinity	Salinity with High % Hatch	Range	Salinity with Low % Hatch	Salinity with No. Hatching
10	20-45	62.3 - 69.5	50 (27.4)	-
15+	10-40	37 - 90	45,50 (0)	45,50
25	15-45	78 - 100	5 (13)	-
35	10-45	69 - 100	5 (0)	5
50	10-45	77.2 - 94.5	5,50 (0)	5,50

+ low fertilization success

TABLE 6. Summary of spotted seatrout 1 day survival data for eggs spawned at different salinities and transferred to 5-50°/$_{00}$S sea water.

Spawning	Salinity with High % Survival	Range	Salinity with Lowest Survival	Salinity with No. Survival
10	15-45	31-50	5,50	5
15+	5,10	76-90	all others	35,40,45,50
25	15-45	46-66	5,50	5,50
35	20-45	25-45.5	5,50	5,50
50	10-45	28-75	5,50	5,50

+ low fertilization success

TABLE 7. Summary of spawning data for Atlantic croaker held at various salinities for 1-3 months. Means with the same letter are not significantly different.

Spawn Salinity	# of Replicate Spawns	# Female/ # Males	# Eggs Collected Per kg Female[1]	Fertilized Egg diameter (μm)[1]	Percent Fertilization[1]	% of Fertilized Eggs Hatched[1]
5	1	3/3	80,000[A]	819.6 ± 6.7[2A]	0[A]	-
15	3	2-3/3	462,890 ± 28,948[A]	765.4 ± 6.9[A]	28.8 ± 1.9[B]	59.6 ± 5.8[B]
25	4	2-3/3	768,429 ± 131,486[A]	763.7 ± 5.8[A]	83.8 ± 2.9[C]	61.3 ± 5.7[B]
35	3	1-3/1-3	232,980 ± 61,319[A]	731.6 ± 5.1[A]	87.8 ± 2.8[C]	73.9 ± 4.2[B]
45	3	2-3/1-3	365,150 ± 117,045[A]	684.5 ± 5.8[A]	9.3 ± 2.6[B]	0[A]

[1] $\bar{x}$ ± S.E.M.

in the spawning salinity was variable, the $35^{o}/_{oo}$ group appeared to exhibit the highest percent hatch while none of the eggs fertilized at $45^{o}/_{oo}$ survived to hatch.

The percent hatch of the total number of eggs spawned at the various salinities and transferred to a range of salinities ($5^{o}/_{oo}$ to $50^{o}/_{oo}$) before the gastrula stage was also examined (Fig. 7). Eggs spawned at $35^{o}/_{oo}$ had higher hatching rates at the various transfer salinities than eggs spawned at the other salinities. Lowest hatching rates were found with eggs spawned at $5^{o}/_{oo}$. In general the transfer salinity did not exert a marked effect on hatching success except at the lowest and highest salinities ($5^{o}/_{oo}$ and $50^{o}/_{oo}$). In addition,eggs transferred to $5^{o}/_{oo}$S took longer to hatch, up to twice as long as those transferred to higher salinities. One spontaneous spawn was obtained at $25^{o}/_{oo}$ salinity without LHRHa injection. The hatching rate of these eggs was not higher than those obtained by hormonal treatment.

Survival of larvae up to the end of the yolk-sac stage, approximately 3 days, was very low below $10^{o}/_{oo}$ and above $25^{o}/_{oo}$ salinities; regardless of spawning salinity (Fig. 8). In general survival was highest at $15^{o}/_{oo}$ salinity. In addition the percentage of abnormal larvae at one day post hatch was higher at the lower salinities (Tables 8 and 9). Typical abnormalities included pericardial edema, lordosis and dorsal caudal curvature (Fig. 9).

It is concluded that croaker are able to spawn successfully at a fairly narrow range of salinities (Fig. 10). No viable eggs were produced at $5^{o}/_{oo}$ and $45^{o}/_{oo}$ salinities and percent fertilization was also greatly reduced at $15^{o}/_{oo}$ salinity. High hatching rates were obtained at $25^{o}/_{oo}$ and $35^{o}/_{oo}$ salinity. However the survival data suggest that survival of the yolk sac larvae is optimal at lower salinities with an optimum around $15^{o}/_{oo}$. Yolk sac larvae had

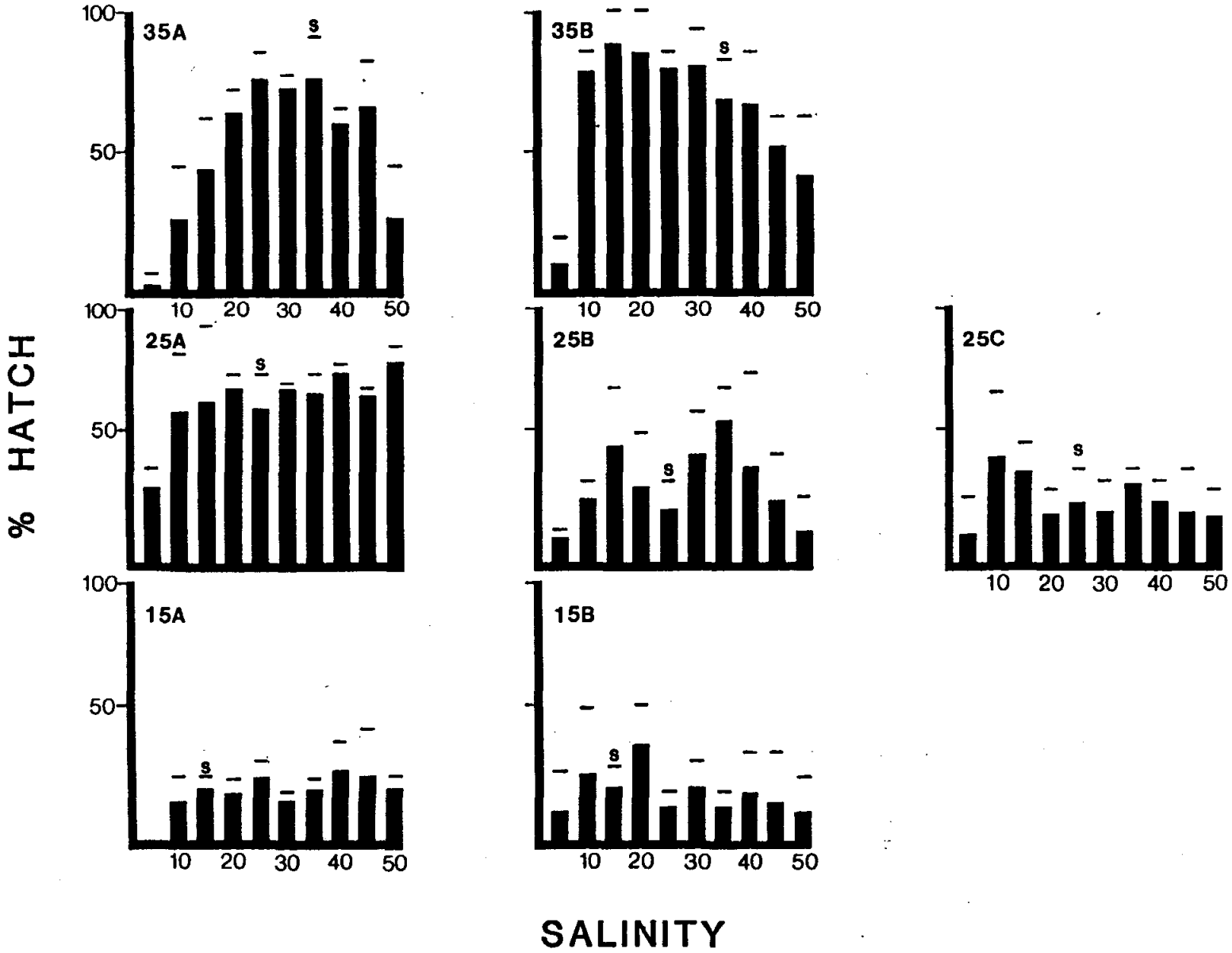


Figure 7. Percent hatch of all Atlantic croaker eggs spawned at test salinities and transferred to various salinities (5o/oo-50o/oo) before the gastrula stage. Bars represent standard deviations and S denotes the fertilization salinity.

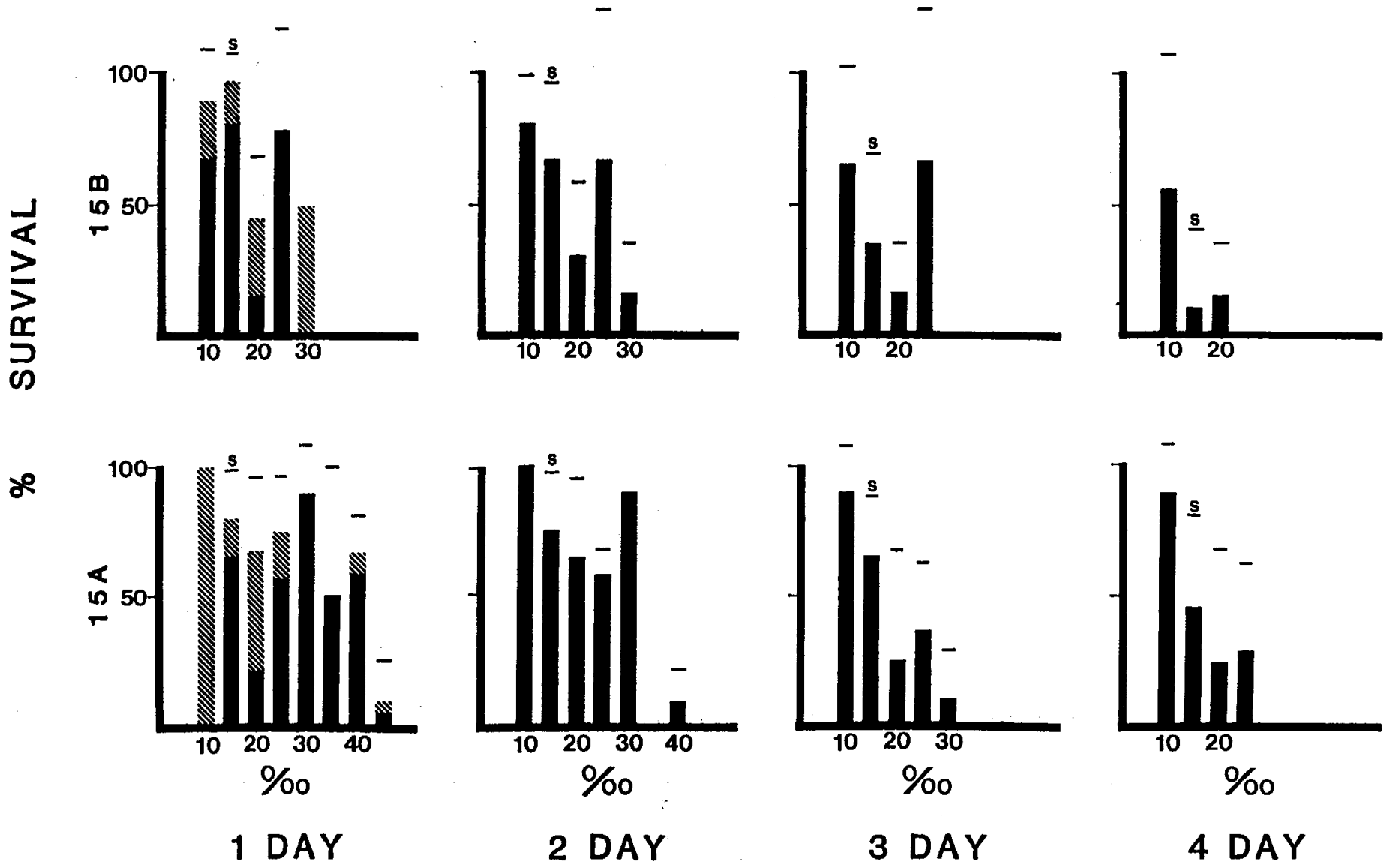


Figure 8. Percent survival of Atlantic croaker larvae spawned at test salinities and transferred to various salinities (5o/oo-50o/oo) before the gastrula stage. Shaded area represents the portion of larvae exhibiting abnormalities. Bars represent standard deviations and S represents fertilization salinity.

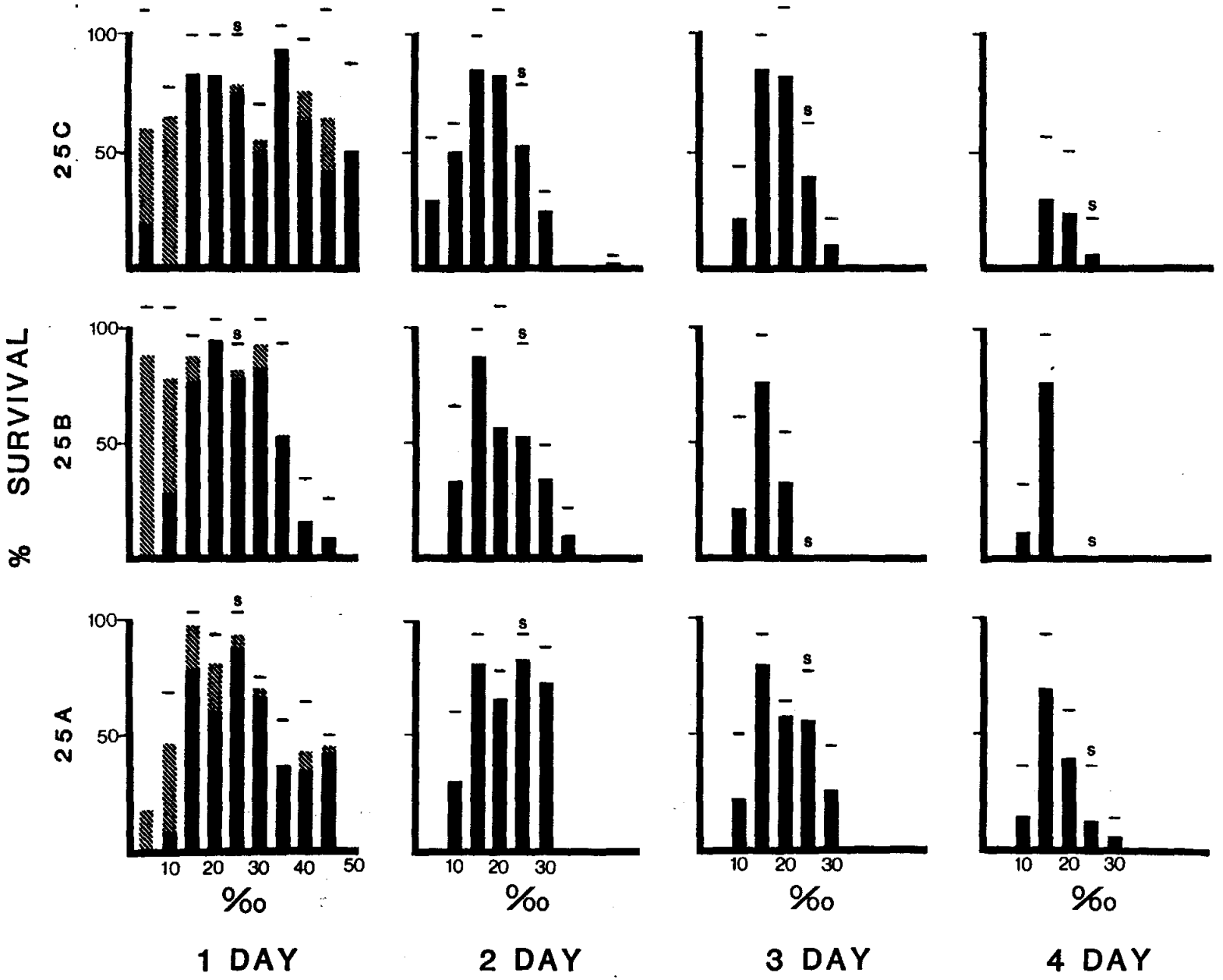


Figure 8. Continued

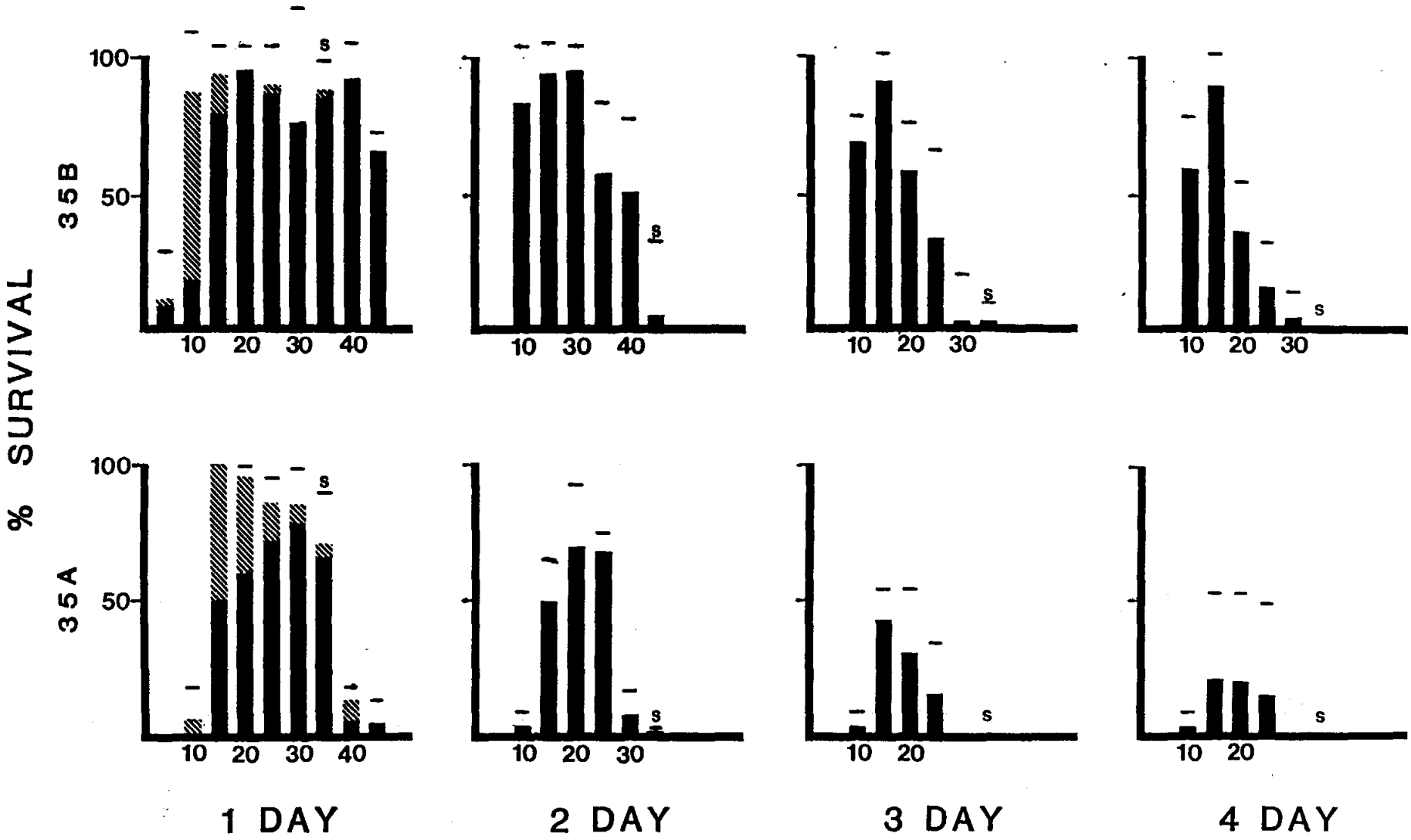


Figure 8. Continued

TABLE 8. Percentage of larval exhibiting abnormalities at 24 hours post-hatch ($\bar{x}$ ± S.E.M.). Salinities represent the conditions in which eggs were spawned and incubated.

Salinity (ppt)	Seatrout	Croaker
5	NM	No Fertilization
10	69.9 ± 8.0	NM
15	38.9 ± 16.2	15.6 ± 4.7
25	26.6 ± 5.8	4.9 ± 1.7
35	1.0 ± 1.0	4.4 ± 1.6
45	NM	No Hatching
50	50.0 ± 50.0*	NM

* Small sample size

NM Not measured

TABLE 9. Percentage of abnormal trout larvae at 24 hours post-hatch after incubating at various salinities ($\bar{x}$ ±S.E.M.). Boldface numbers represent larvae fertilized, hatched and incubated in test salinity.

Incubation Salinity	SPAWN SALINITY 10	15	25	35	50
5	*	100 ± 100	0 ± 0	*	*
10	69.9 ± 8.0	43.3 ± 6.7	16.3 ± 3.8	*	100 ± 100
15	27.8 ± 23.7	38.9 ± 16.2	2.3 ± 2.3	0 ± 0	20.9 ± 8.5
20	41.7 ± 22.0	50.0 ± 50.0	37.7 ± 13.5	20 ± 20	10.2 ± 6.5
25	25 ± 14.4	33.3 ± 33.3	26.6 ± 5.8	*	0 ± 0
30	4.8 ± 4.8	66.7 ± 33.3	*	2.1 ± 2.1	11.9 ± 11.9
35	0 ± 0	*	14.8 ± 8.1	1.0 ± 1.0	15.0 ± 7.7
40	0 ± 0	*	11.4 ± 7.3	8.4 ± 8.4	71.7 ± 9.2
45	0 ± 0	*	8.3 ± 8.3	0 ± 0	100 ± 100
50	0 ± 0	*	11.1 ± 11.1	*	50.0 ± 50.0

* No live larvae remaining at 24 hours post-hatch.

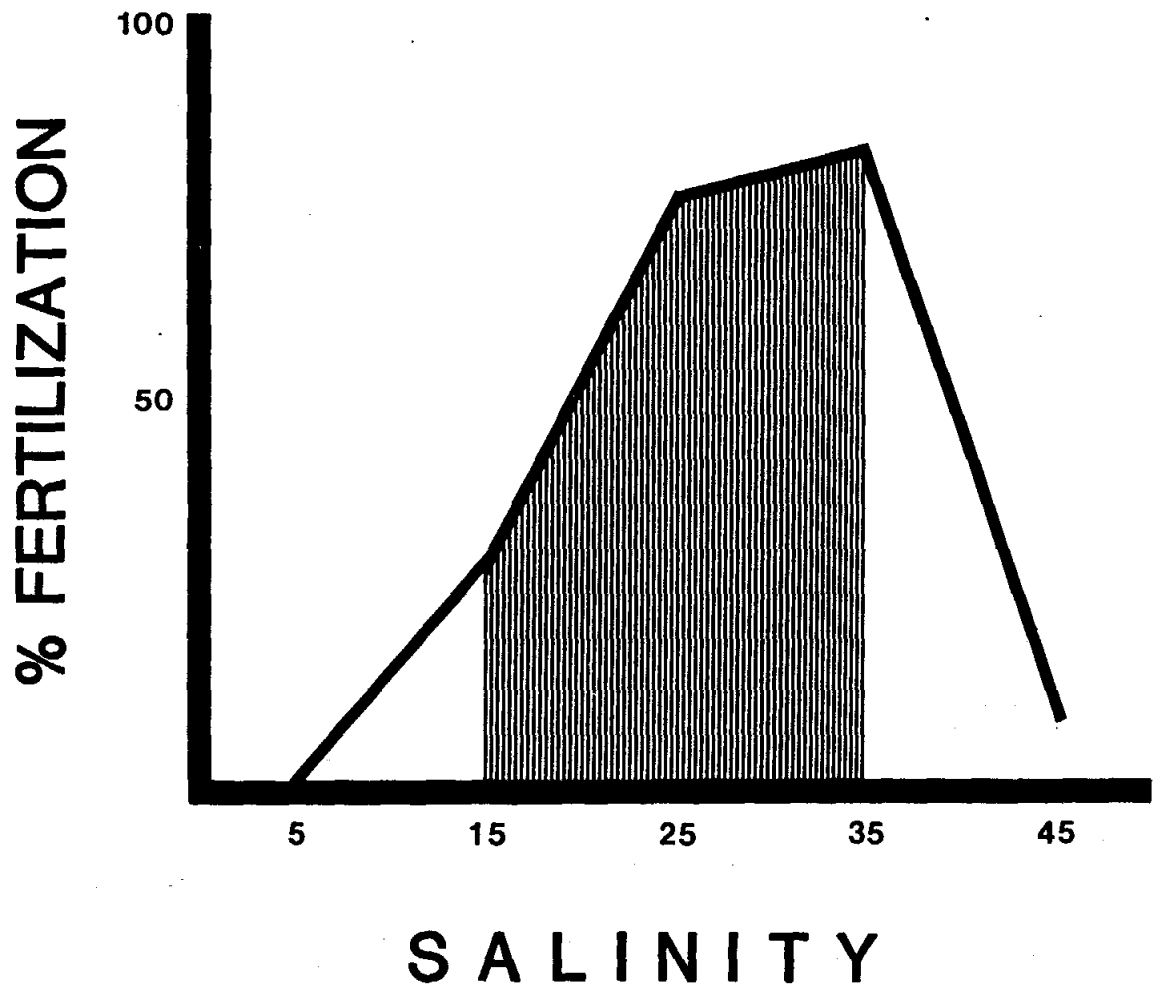


Figure 10. Percent fertilization for Atlantic croaker eggs at test salinities. Shaded area represents salinities where eggs hatched.

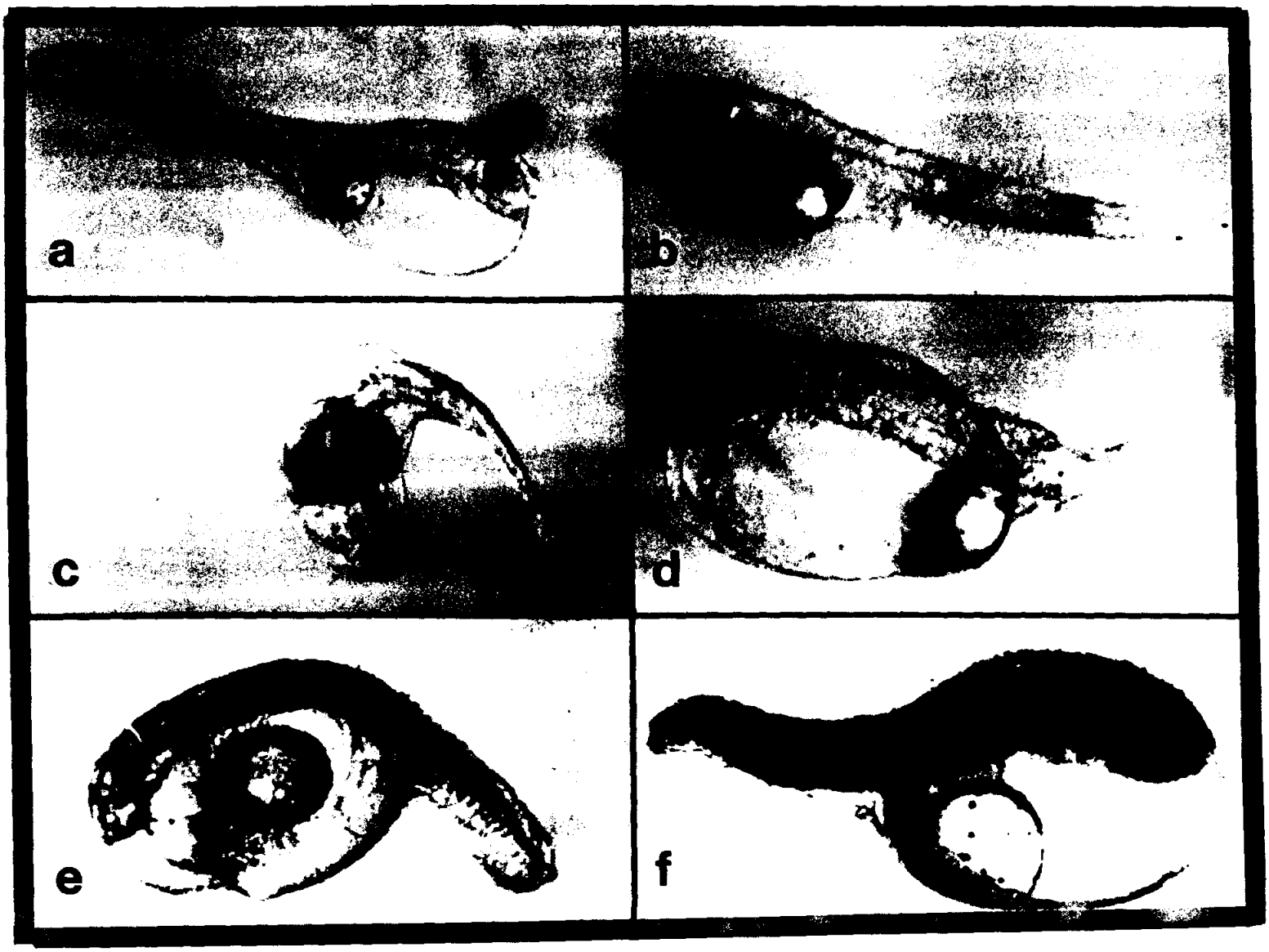


Figure 9. Appearance of Atlantic croaker larvae hatched in low salinity ($5^{o}/_{oo}$S-$15^{o}/_{oo}$S) sea water a-b - normal larva (b - approximately 48 hrs post-hatch); c-f - abnormal larva with pericardial edema (d-f) lordosis, (d-f), dorsal caudal curvature (d,e).

narrower limits for salinity tolerance than the fertilized eggs, in general 3-day survival was only high in the salinity range of $10^{o}/_{oo}$ to $20^{o}/_{oo}$ or $25^{o}/_{oo}$.

DISCUSSION

1. Reproductive endocrinology and ovarian growth.

The present results show that salinity can exert a marked effect on ovarian growth in spotted seatrout. Unfortunately equivalent information for other species is largely lacking. Salinity effects on gonadal activity have only been investigated in a few species (Lam, 1983). Lam and Munro (1987) demonstrated that the growth rate of successive cohorts of eggs in the ovaries of threespine sticklebacks, and hence the spawning interval, was influenced by the ambient salinity. In the present study ovarian growth was significantly impaired in spotted seatrout at low and high salinities. Spotted seatrout is a multiple spawning species in South Texas (Brown-Peterson *et al.*, 1988). Therefore it is possible that suboptimal salinities result in reduced spawning frequency in spotted seatrout leading to a reduction in total annual fecundity. A marked decrease in the production of eggs and larvae could lead to a population decline. It is therefore important to investigate the effects of salinity on spawning frequency in spotted seatrout.

Salinity effects on ovarian growth were most evident after one month exposure to salinity extremes. Most fish were able to complete oocyte and ovarian growth after two months exposure to high and low salinities. These results suggest that acclimation to salinity extremes may occur during chronic exposure. Other euryhaline teleosts, the mullets, do not normally produce vitellogenic eggs in freshwater (Abraham *et al.*, 1966) but reproduction is normal if they are transferred to fresh water as fry and reared to maturity

there (Eckstein and Eylath, 1970). Similarly, spotted seatrout inhabiting hypersaline and hyposaline habitats may have different salinity tolerances with regard to reproduction which may have an environmental basis. The differences in salinity tolerances exhibited by spotted seatrout from different estuaries, if they do indeed exist, as well as the relative importance of environmental and genetic factors in these differences, are important topics for future study with regard to long term changes in fresh water inflow.

Circulating levels of estradiol were significantly decreased in spotted seatrout after one month exposure to extreme salinities. To our knowledge this is the first report of alterations in the reproductive endocrinology of any marine or euryhaline teleost due to salinity changes. Salinity effects on gonadal growth appear to be mediated by changes in circulating levels of reproductive hormones. There was a close association between decreased plasma estradiol concentrations and ovarian and oocyte growth. Estradiol has been shown to regulate vitellogenesis and thereby influence ovarian growth in this species (Smith and Thomas, in press). On the other hand testosterone is a major steroid in female fishes, but its precise function is still uncertain. The changes in fish reproductive physiology are probably exerted at the hypothalamic level. A wide variety of stressors, including pollutants, are known to act at the brain to alter reproductive function in vertebrates. The reproductive dysfunction in spotted seatrout can therefore be explained solely on the basis of salinity acting as a nonspecific stressor. However, a specific salinity-dependent effect may also be involved. The pituitary hormone prolactin may play an important role in fish reproduction (Mazzi and Vellano, 1987). Moreover, we have shown that the activity of prolactin cells in red drum is profoundly influenced by the ambient salinity (Yan and

Thomas, 1988). Additional studies will be required to determine the mechanisms by which salinity changes disrupt reproductive physiological function in teleosts.

Our results suggest that the reproductive endocrine control of oocyte and ovarian growth may be most easily disrupted at early stages of ovarian recrudescence. Salinity had only minor affects on plasma estradiol levels in Atlantic croaker at an advanced stage of recrudescence. Unfortunately we were unable to collect 2-year old croaker at an earlier stage of recrudescence. It would be interesting to repeat these experiments with juvenile croaker to determine whether long term exposure impairs sexual maturation and early stages of oocyte growth in this species.

Only minor effects of salinity on reproductive physiological function in male spotted seatrout were observed. We have previously found that there is a sex difference in the sensitivity of spotted seatrout reproduction function to physical stressors (Safford and Thomas, 1987). Thus reproductive endocrine function in females appears to be much more susceptible to environmental perturbations.

2. Spawning and Hatching Success.

An effect of adult acclimation salinity on fertilized egg diameter and neutral buoyancy in a static test was observed in Atlantic croaker in this study. Adults acclimated to high salinities produced smaller and less buoyant eggs than fish acclimated to lower salinities. A similar effect of salinity on egg size was found in spotted seatrout. This size and buoyancy increase has been attributed to increased water absorption during final oocyte maturation and water hardening at low salinities (Solemdal, 1967, Craik and Harvey, 1987). However, only partial buoyancy compensation occurred at low salinities so that a majority of the fertilized croaker eggs produced at $15^{o}/_{oo}$ sank at salinities below than $25^{o}/_{oo}$. Holt

et al. (1981) reported that red drum eggs fertilized at $26^{o}/_{oo}$-$32^{o}/_{oo}$ by fish acclimated to those salinities sank in salinities less than $25^{o}/_{oo}$. Although it is likely that fertilized pelagic sciaenid eggs would remain in the water column in salinities moderately lower than $25^{o}/_{oo}$ by turbulence, salinities much lower than this would constitute a serious egg buoyancy limitation for croaker. Croaker eggs which sink to the bottom would not be dispersed and would probably encounter conditions unfavorable for survival such as low dissolved oxygen, deleterious microorganisms and predators.

Our results showed that spotted seatrout eggs could be fertilized over a much wider salinity range ($10^{o}/_{oo}$-$50^{o}/_{oo}$) than croaker ($25^{o}/_{oo}$-$35^{o}/_{oo}$). Thus fertilization success appears to be a limiting factor for croaker in both low and high salinities but only in high salinities for seatrout. May (1975) and Palmer and Able (1987) demonstrated that while sperm were generally motile for a longer in low salinities ($5^{o}/_{oo}$-$15^{o}/_{oo}$), motility increased with increasing salinity. Gwo (personal communication) found that motility of sperm from spotted seatrout and Atlantic croaker was maximum between $18^{o}/_{oo}$ and $30^{o}/_{oo}$ salinity. In addition in the present study it was noted that male seatrout acclimated to $50^{o}/_{oo}$ and male croaker at $45^{o}/_{oo}$ did not consistently have free flowing milt. Salinity effects on female gametes may also contribute to the lower fertilization success at salinity extremes. Able and Palmer showed that ovarian and plasma osmolality in *Fundulus heteroclitus* are different from that of the fertilization medium at extreme salinities ($5^{o}/_{oo}$ and $45^{o}/_{oo}$), and proposed that when ovulated oocytes are released into extreme salinities there is a potential for osmotic shock-induced damage to the egg (Able and Palmer, 1988; Palmer and Able, 1987). Therefore the reduced fertilization observed in extreme salinities for seatrout and croaker may be due to a combination of reduced sperm motility/longevity and osmotic shock to the oocytes.

Hatching success for seatrout eggs was only significantly impaired at 50‰ salinity and was high over the range of 10‰ to 35‰. Salinities between 35‰ and 50‰ were not investigated in the present study so the upper limit for high hatching success is not known. In contrast, hatching in croaker eggs was limited to 15‰-35‰, a much narrower range. Moreover there was a tendency for croaker eggs incubated at 5‰ to have a prolonged hatching time and reduced hatching success which was independent of fertilization salinity. Altered incubation time and reduced hatching success at extreme salinities, especially low salinities, have previously been reported for other teleost species (May, 1975; Holliday, 1960; Holt *et al.*, 1981; Forrester and Alderdice, 1966; Alderdice and Forrester, 1971). Holt *et al.* (1981) demonstrated that red drum eggs fertilized at 26‰-32‰ had poor hatching success when transferred to 10‰ salinity seawater.

The slightly lower salinity tolerance of seatrout and croaker eggs and larvae observed in these experiments (Fig. 11) compared to those found by Joan Holt and coworkers (see Chapter III) may be due to the developmental stage of the eggs when they are transferred to various incubation salinities. The eggs were transferred earlier in the present study, before the gastrula stage. Lee and Menu (1981) reported that fertilized *Mugil cephalus* eggs at the 2-blastomere stage were less tolerant to salinity change than the later gastrula stage.

Some abnormal larvae were found at all of the spawning and transfer salinities in both species. However, the incidence and severity of abnormalities were greatest at salinities of 5‰ and 10‰. Fewest abnormalities were found in eggs spawned at 25‰ and 35‰. Eggs of the euryhaline teleost *Mugil cephalus* fertilized at 30‰ also produced large numbers of abnormal larvae when incubated at high and low salinities (15‰S and 50‰S,

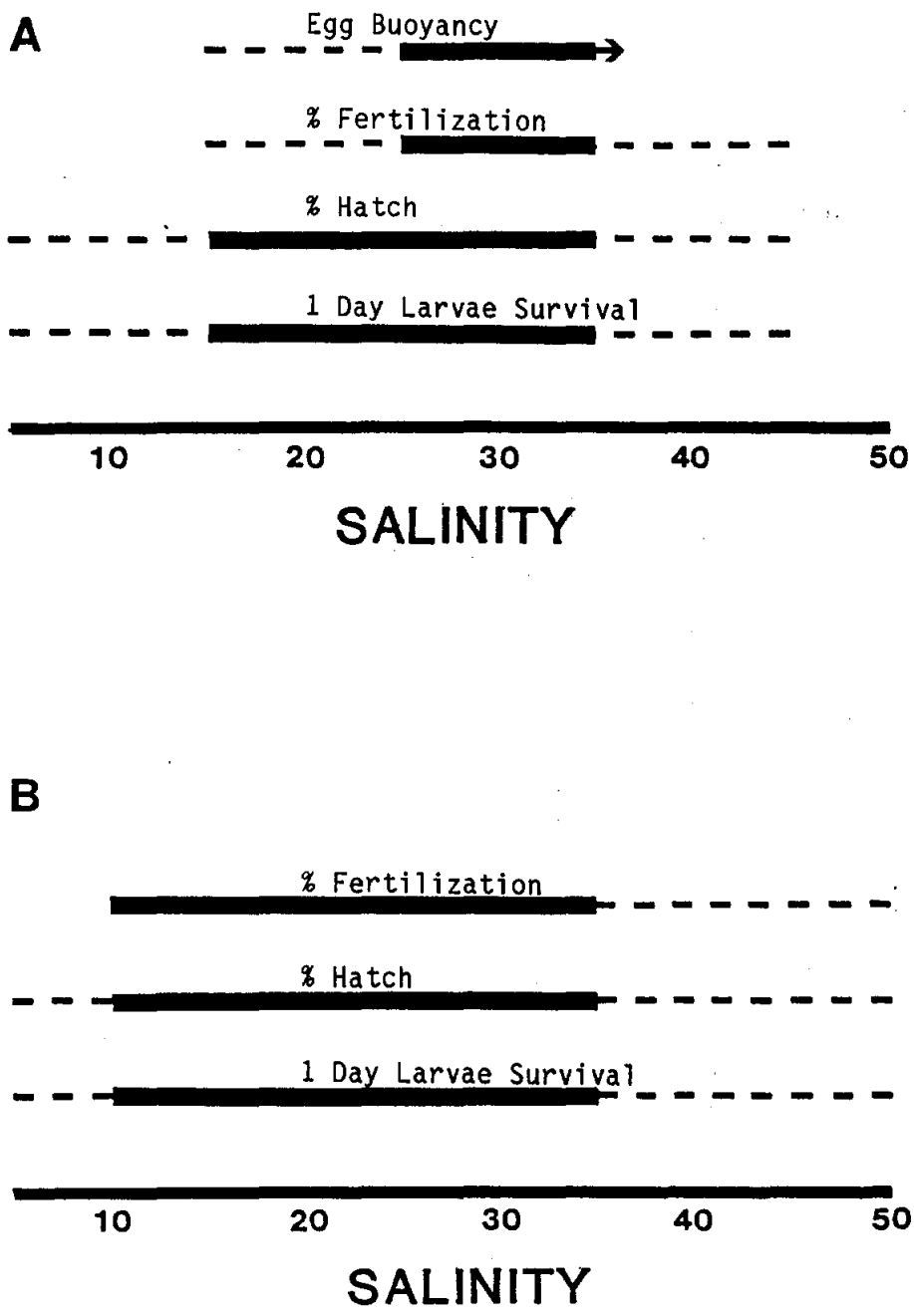


Figure 11. Biological limits for successful reproduction in Atlantic croaker (A) and spotted seatrout (B) at test salinities. Solid bars indicate salinities where acceptable levels were measured. Dashed lines represent uninvestigated salinities between measured acceptable and measured unacceptable levels.

Lee and Menu, 1981). May (1975) found that many *B. icistia* larvae at reared low salinities exhibited varying degrees of edema, which was inversely related to the salinity. Croaker also showed edema in the present study, although no distinct salinity-dependent effect on the severity of edema was noted. Interestingly, the abnormalities observed in croaker larvae after exposure to salinity extremes are similar to those seen after exposure to a polychlorinated biphenyl mixture (Thomas, unpublished).

The one day larval survival for spotted seatrout, although low, indicates a wide range of salinity tolerance ($10^{o}/_{oo}$-$35^{o}/_{oo}$). Survival at $50^{o}/_{oo}$ was significantly reduced so that the upper tolerance limit may be between $35^{o}/_{oo}$ and $50^{o}/_{oo}$. Croaker larvae had a similar low salinity tolerance. Croaker larvae, at salinities greater than $30^{o}/_{oo}$ experienced high mortality. The optimum salinity for croaker larvae, therefore, appears to be between $15^{o}/_{oo}$ and $20^{o}/_{oo}$. Fertilization salinity affected larval survival only at $10^{o}/_{oo}$S, with eggs fertilized at low salinities exhibiting higher survival at $10^{o}/_{oo}$ than those fertilized at higher salinities (Fig. 11).

3. Conclusions

The results of these studies indicate the approximate salinity range for successful reproduction of Atlantic croaker and spotted seatrout. The range of salinities at which natural populations of croaker and seatrout can reproduce may differ somewhat from those reported here, however, due to possible population differences and also the stress associated with maintaining captive broodstock.

Exposure of Atlantic croaker at an advanced stage of ovarian growth to salinity extremes does not impair oocyte maturation except at the highest salinity ($45^{o}/_{oo}$). However croaker apparently have much narrower salinity limits ($25^{o}/_{oo}$-$35^{o}/_{oo}$) for high fertilization

(Fig. 11). High percentages of abnormalities were observed in larvae hatched at low salinities. High rates of hatching only occurred between 15°/$_{00}$ and 35°/$_{00}$S. In addition, larval survival was severely reduced at salinities greater than 30°/$_{00}$. Taken together, these results suggest that reproduction in croaker is limited to salinities of 25°/$_{00}$ to 30°/$_{00}$-35°/$_{00}$.

Reproductive endocrine function and oocyte growth were significantly impaired in spotted seatrout exposed to 45°/$_{00}$ and 20°/$_{00}$ salinity or lower during the early stages of ovarian recrudescence. Seatrout oocytes eventually mature at these salinities, although fecundity is reduced at the high salinity. Spotted seatrout have a much broader salinity range for successful fertilization and hatching (10°/$_{00}$-35°/$_{00}$) than croaker and appear to be limited only at high salinities in the range of 35°/$_{00}$-50°/$_{00}$. These results suggest that although reproduction in spotted seatrout may be optimum around full strength sea water (35°/$_{00}$), they are able to reproduce at broader range of salinities (20-45°/$_{00}$S).

The different life histories of the two species probably account for differences observed. Seatrout inhabit estuaries and bays which can have marked salinity fluctuations for their entire life cycle so it is not surprising that reproduction is successful over a broad range of salinities. In contrast Atlantic croaker live only a portion of their life cycle in bays and estuaries and then migrate to offshore areas to spawn. The only phases of their reproductive cycle where they may experience marked salinity fluctuations are during ovarian recrudescence and post-larval development. Other stages of croaker reproduction, such as fertilization and hatching, normally occur in full strength sea water and are not as tolerant to salinity extremes.

ACKNOWLEDGEMENTS

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II. SALINITY TOLERANCE IN LARVAE OF SPOTTED SEATROUT, RED DRUM AND ATLANTIC CROAKER

MATERIAL AND METHODS

Eggs were collected using different methods for the three species. Atlantic croaker were collected as they migrated offshore in the fall, held in the lab and injected with hormones when eggs were mature (either 150 IU HCG per kg fish or 0.1 mg per kg fish of an analogue of LHRH, Thomas and Boyd, 1988). Spotted seatrout were captured with a gill net, at dusk during the spawning season, and gametes from "running-ripe" fish were either dry fertilized or stripped into beakers of ambient or test salinity water before fertilization. Eggs and larvae from spotted seatrout collected from two sites (1) Copano Bay, 24 ppt and (2) Aransas Bay, 32 ppt were compared. A third site in the Laguna Madre with 47 ppt water was sampled (Fig. 12) and many large spotted seatrout were collected but the females were not in spawning condition although the males were producing free flowing sperm.

Spawning stocks of all three species were maintained in the laboratory under controlled temperature and day length conditions. Eggs were collected from tanks when fish spawned in response to seasonal temperature and photoperiod manipulations (Arnold *et al.*, 1976). The laboratory temperature was maintained at reported optimum spawning conditions for each species. This was 28° C for spotted seatrout (Arnold *et al.*, 1976; Wohlschlag and Wakeman, 1978) and red drum (Arnold *et al.*, 1977) and 24° C for Atlantic croaker (D.E. Hoss, NMFS Beaufort, N.C., personal communication).

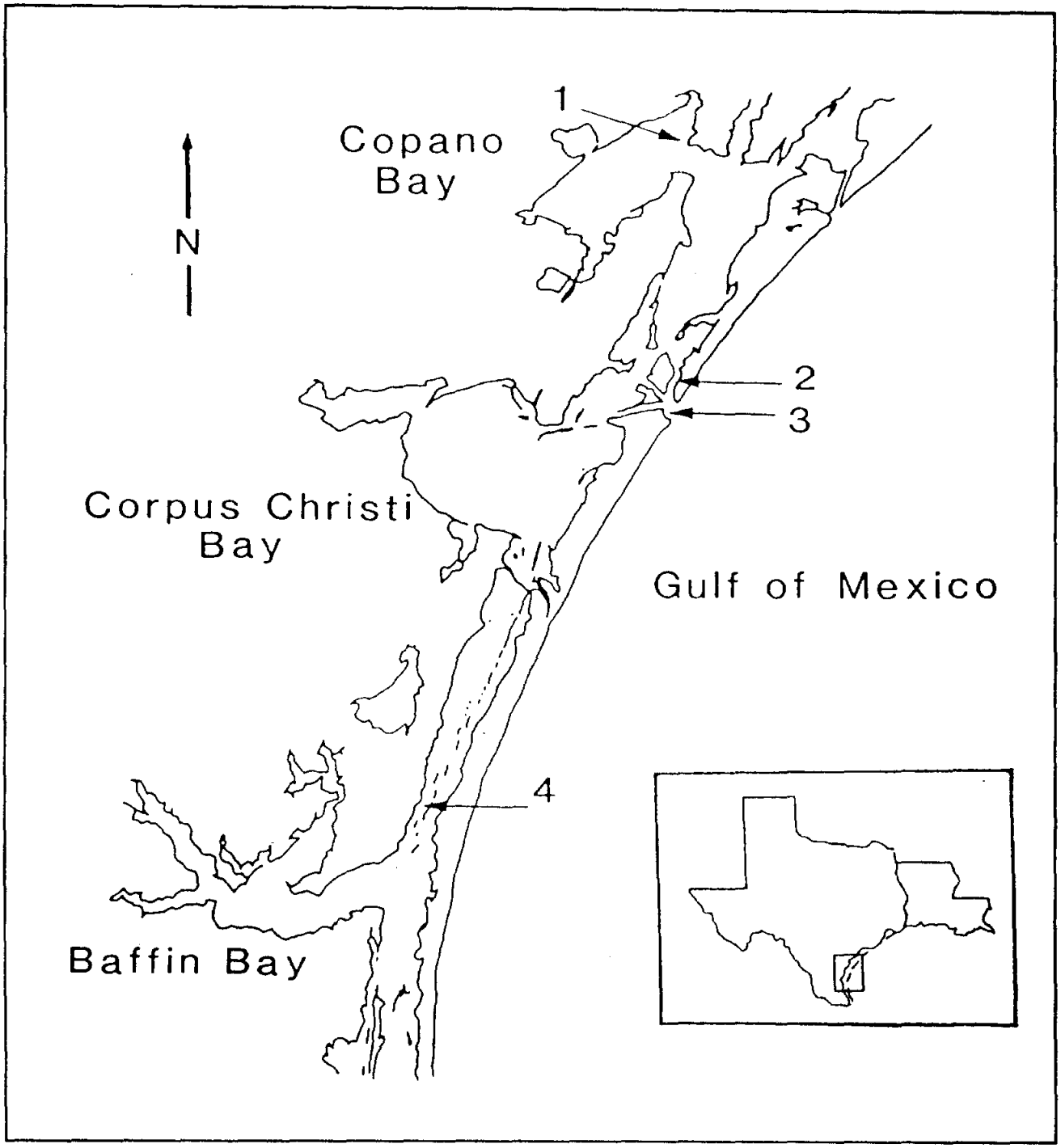

Figure 12. Collection sites for adult spotted seatrout. 1. Copano Bay, 2. Aransas Bay, 3. Laboratory broodstock, 4. Laguna Madre.

Egg hatch rate and 3 day survival tests followed methods described in Holt *et al.* (1981). For long-term tests, larvae were cultivated in 150 liter conical tanks (61 cm diameter, 91 cm height) with internal biological filters which eliminated the need for water exchanges (Craig *et al.*, in press). Larvae were fed rotifers *Branchionis plicatilis* at a density of 3-5 per ml of water from the second to the twelfth day after hatching. Their diet was changed to brine shrimp nauplii (*Artemia* sp.) at 1-3 per ml from day 10 to day 21, followed thereafter by finely minced shrimp.

Acute (18 hour) salinity tolerance tests were conducted for all three species according to procedures described by Yin and Blaxter (1987).

Shock treatment tests involved larvae hatched and reared in spawning salinity water and then transferred directly into the test salinity. Larvae were subjected to a range of test salinities from 0 to 56 ppt (intervals of 4-8 ppt; smaller intervals were employed at critical points) using one liter glass beakers, fitted with nitex mesh baskets and aerated by capillary tubes outside the basket.

Additionally, the following two protocols were used to test for acclimation in spotted seatrout larvae using the survival results from the shock treatment tests as controls.

Hypersaline acclimation treatment test involved larvae transferred from the spawning salinity (32 ppt) into hypersaline water (with respect to spawning salinity) over a 6 day period at a rate of 2 ppt every second day. Larvae were reared in the hypersaline water and were subjected to the upper half of the salinity treatment range used in the shock treatment test. Acclimation salinity was the lowest salinity tested.

Hyposaline acclimated treatment test involved larvae reared from the egg stage in 20 ppt salinity. These larvae were subjected to the lower half of the salinity treatment range used in the shock treatment test.

Three replicate tests were made at each salinity treatment every other day for nine days. Larvae for each replicate were chosen randomly from independent replicate holding tanks. From 10 to 15 larvae were transferred to each test beaker along with 10 ml of holding tank water. Percentage survival was assessed by counting the number of survivors after an eighteen hour period. Eighteen hours was chosen and verified as an appropriate time to test for salinity stress tolerance without complication by hidden parameters such as starvation, and unionized ammonia buildup.

Statistical Analysis

Probit analysis was performed on the response criteria following the methods described in Yin and Blaxter (1987). LD_{50} values with associated fiducial limits were obtained using PROC PROBIT, part of Base SAS software (SAS, 1982) on an IBM mainframe computer rather than the Hewlett & Plackett package used by Yin and Blaxter. The PROBIT procedure requires inclusion of three variables: dose, subjects and response. Treatment salinities were transformed such that the salinity at spawning represented a dose of 0 with the other treatments (doses) being assigned values proportional to the magnitude of their difference from the spawning salinity. This enabled appropriate implementation of the PROBIT procedure default which assigns control status to observations with a dose of 0. The number of larvae transferred to the test represented the test subjects and the number of survivors represented the response.

Arc-sine, square-root transformations of the percentage survival data for control, hypersaline and hyposaline acclimated larvae were analyzed using ANOVA in the General Linear Model of the Statistical Analysis System (SAS). Tukey's multiple range test was used to identify significant differences.

RESULTS AND DISCUSSION

A. Egg hatch rate and three day survival

Successful egg hatching in all three species occurred over a broad range of salinities (10 to 55 ppt) when eggs were transferred after formation of the embryonic axis (12 hr postfertilization; 25-27° C). Results for spotted seatrout, red drum and Atlantic croaker are presented in Figures 13, 14 and 15 respectively. A notably broader range of tolerance for spotted seatrout compared to red drum and Atlantic croaker was demonstrated by the early survival data.

The salinity range in which normal egg development occurred was greatly reduced in eggs transferred before or during early cleavage. For example, spotted seatrout eggs transferred immediately after dry fertilization showed no development in 0 and 10 ppt, developed only to the blastula stage in 20 ppt and developed normally in 30 and 40 ppt water. Similar increased tolerance for more mature embryos was demonstrated for grey mullet (*Mugil cephalus* L.) where eggs transferred at the gastrula stage were more tolerant to salinity change than were those transferred at the 2-blastomere stage (Lee and Menu, 1981).

Spotted seatrout eggs and milt were stripped from spawning adults captured in 24 ppt from Copano Bay and 32 ppt from Aransas Bay (Fig. 12). Although fish in spawning condition were not found in Laguna Madre at 47 ppt, the state of the gonads suggested that spotted sea trout had spawned in the recent past, perhaps when saline conditions were lower. Mean diameters of eggs varied with spawning salinity. The smallest eggs (0.6 mm) were from fish collected at 32 ppt and larger eggs (0.7 mm) from 24 ppt salinity. A similar relationship was seen in eggs spawned in the lab from adult spotted seatrout subjected to salinity from 22 to 35 ppt (Table 10).

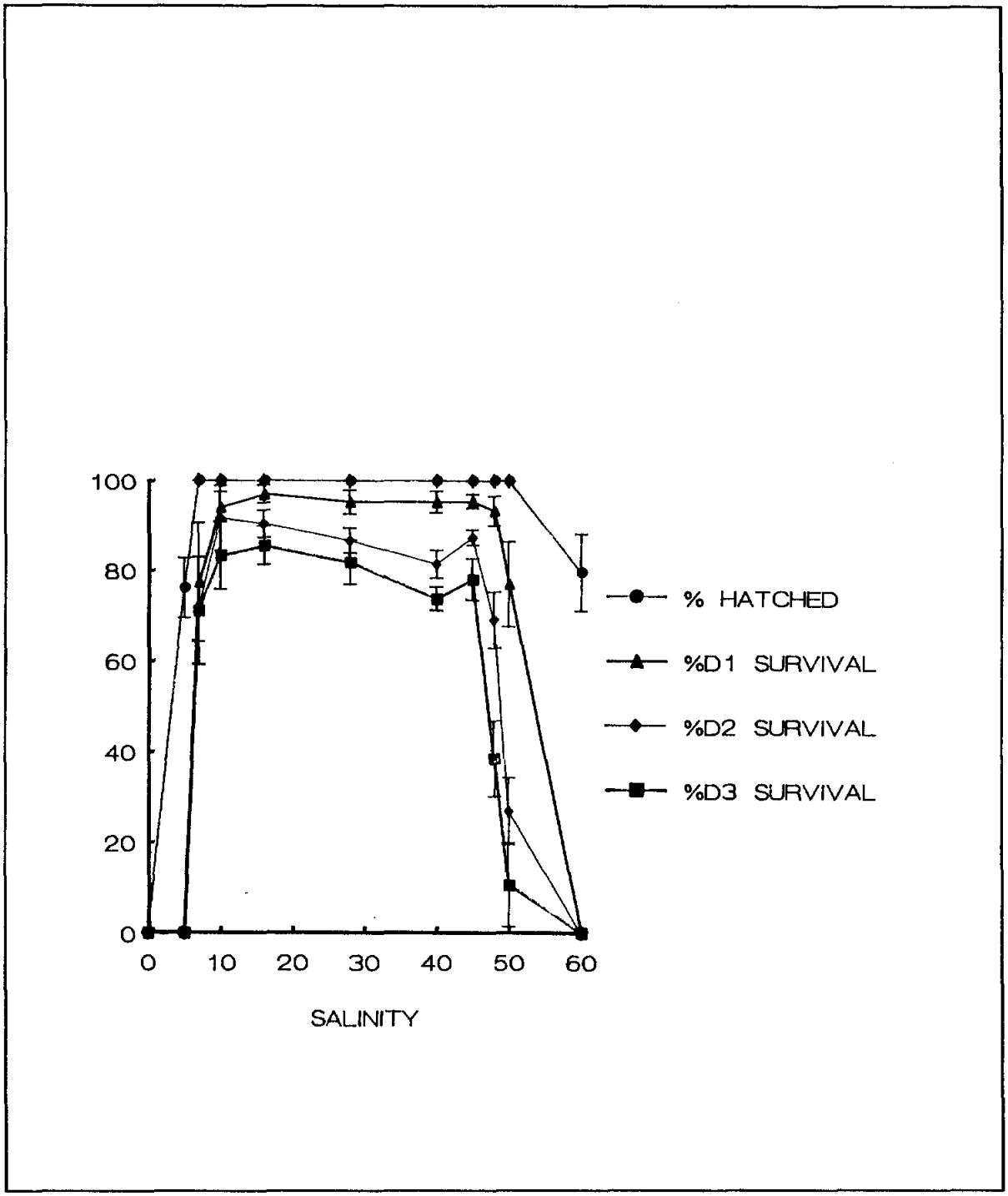

Figure 13. Egg hatch rate and early larval survival for spotted seatrout. Error bars indicate one standard error.

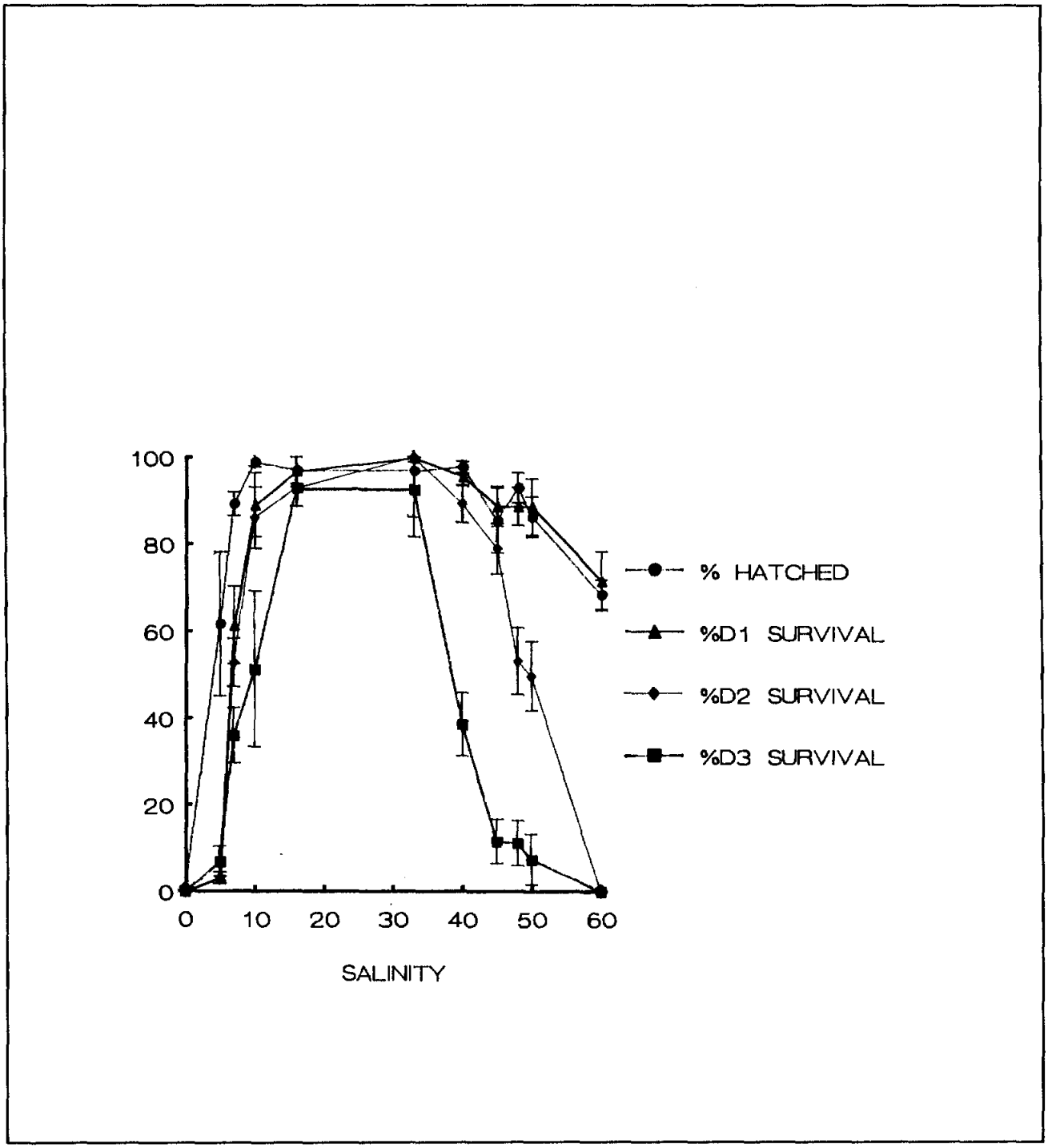

Figure 14. Egg hatch rate and early larval survival for red drum. Error bars indicate one standard error.

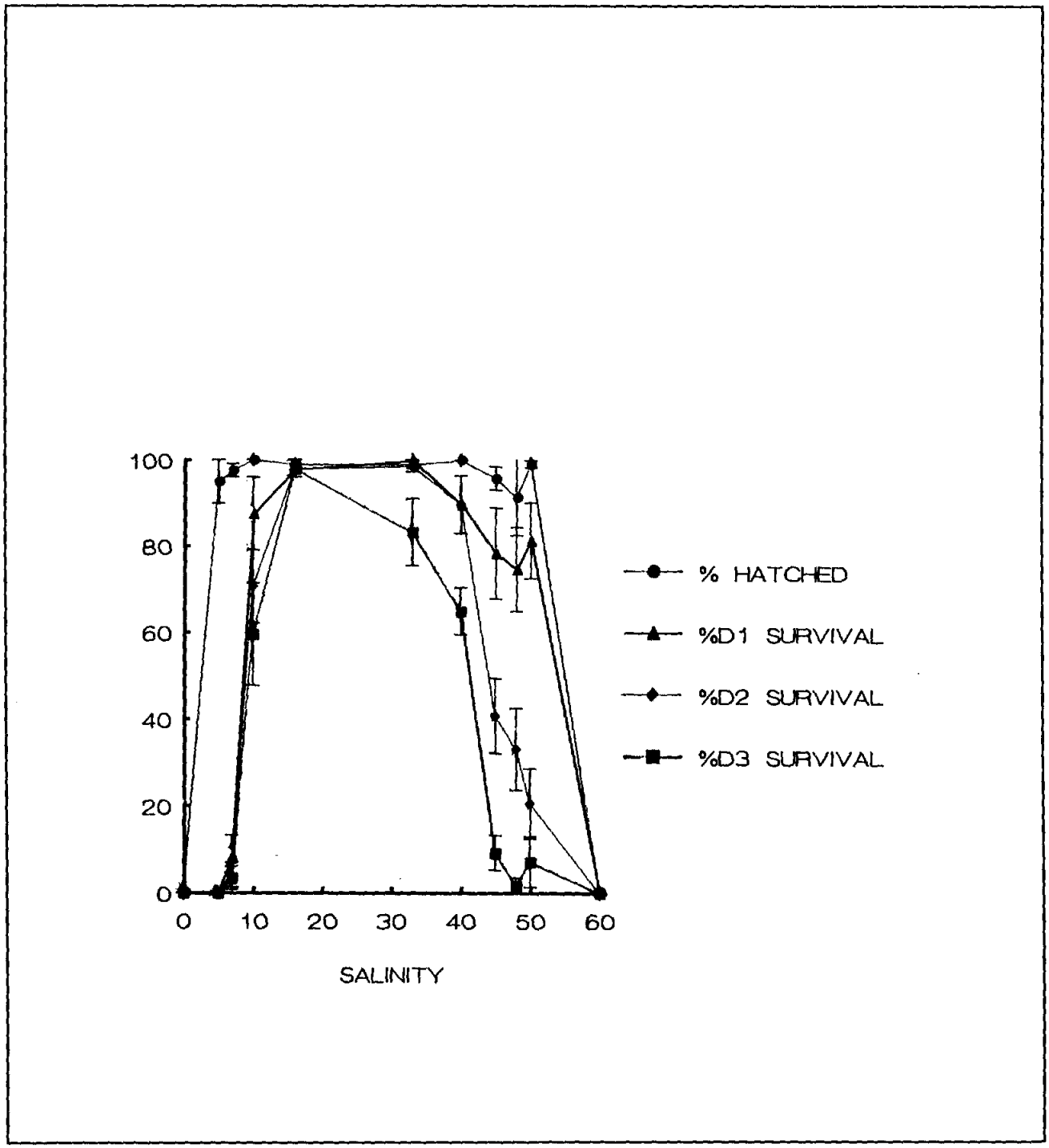

Figure 15. Egg hatch rate and early larval survival for Atlantic croaker. Error bars indicate one standard error.

TABLE 10. Size of spotted seatrout eggs from laboratory spawns of fish subjected to gradual salinity changes ($\leq$ 4 ppt d^{-1}) during the simulated spawning season.

Egg Diameter $\bar{x}$	Egg Diameter (S $\bar{x}$)	Salinity ppt	Spawn Date
0.83	(.006)	19	4/23/89
0.82	(.004)	22	3/31/89
0.81	(.006)	24	11/09/88
0.76	(.007)	28	2/16/89
0.75	(.008)	30	10/18/88
0.70	(.014)	32	9/20/88
0.71	(.014)	35	10/24/88

However, diameters of newly fertilized eggs transferred directly into test salinities, were the same at all salinities. This is contrary to expectations that volume regulation at, or right after fertilization would change egg size and buoyancy. Wild spotted seatrout eggs have been collected in south Texas bay water ranging from 19 to 36 ppt salinity (Holt *et al.*, 1988).

B. Long-term growth studies

In two week old Atlantic croaker, we found larvae tolerated salinities as low as 15 ppt but only larvae greater than 10 mm survived at 5-45 ppt. Red drum survived from hatching to two weeks and grew equally well in 15-30 ppt water (Holt *et al.*, 1981). In fact, red drum are routinely raised in 10 ppt water after they reach a length of 5 mm (approximately two weeks old), and they can be transferred directly to freshwater at 15 mm SL (Crocker *et al.*, 1981).

Spotted seatrout larvae tolerated changes of 4 ppt per day from 28 ppt spawning salinity to 16-45 ppt salinity. Three week growth rates were significantly better in 16 than in 28 ppt on 45 ppt salinity ($p < .05$) (Fig. 16, Table 11). Poor growth in high salinity (45 ppt) occurred during the first two weeks but during the third week, growth rate of fish in 45 ppt increased dramatically. Although spotted seatrout larvae tolerate 45 ppt, slower growth during the early stages retards development and increases the length of the larval stage and the coincident potential for high mortality rates due to external causes.

C. Acute salinity tolerance

Daily changes in osmoregulatory ability were investigated by evaluating short-term survival of larvae subjected to acute salinity changes. Yolk-sac larvae of spotted seatrout were more tolerant of extreme salinities than larvae at age 3 days (Figure 17). Reduced tolerance on day 3, noted regularly in acute salinity tests, occurred when larvae had well developed eyes and mouth and had begun to feed (critical period). Integumentary osmoregulatory structures (ie. chloride cells) may not be sufficient to handle the high hydromineral load taken in with food and water at first feeding when osmoregulatory organ systems have not developed sufficiently. This hypothetically reduced osmoregulatory ability is overcome by day 5 as their salinity tolerance increased daily after this critical period.

Fifty percent survival values (LD_{50}) confirmed these age dependent changes in salinity tolerance (Figure 18). Values have differences defined by 95% fiducial limits in all cases except day nine in the lower salinity tolerance. Low tolerance on day three (critical period), is clearly demonstrated as well as a broadening of the range tolerated for days tested thereafter.

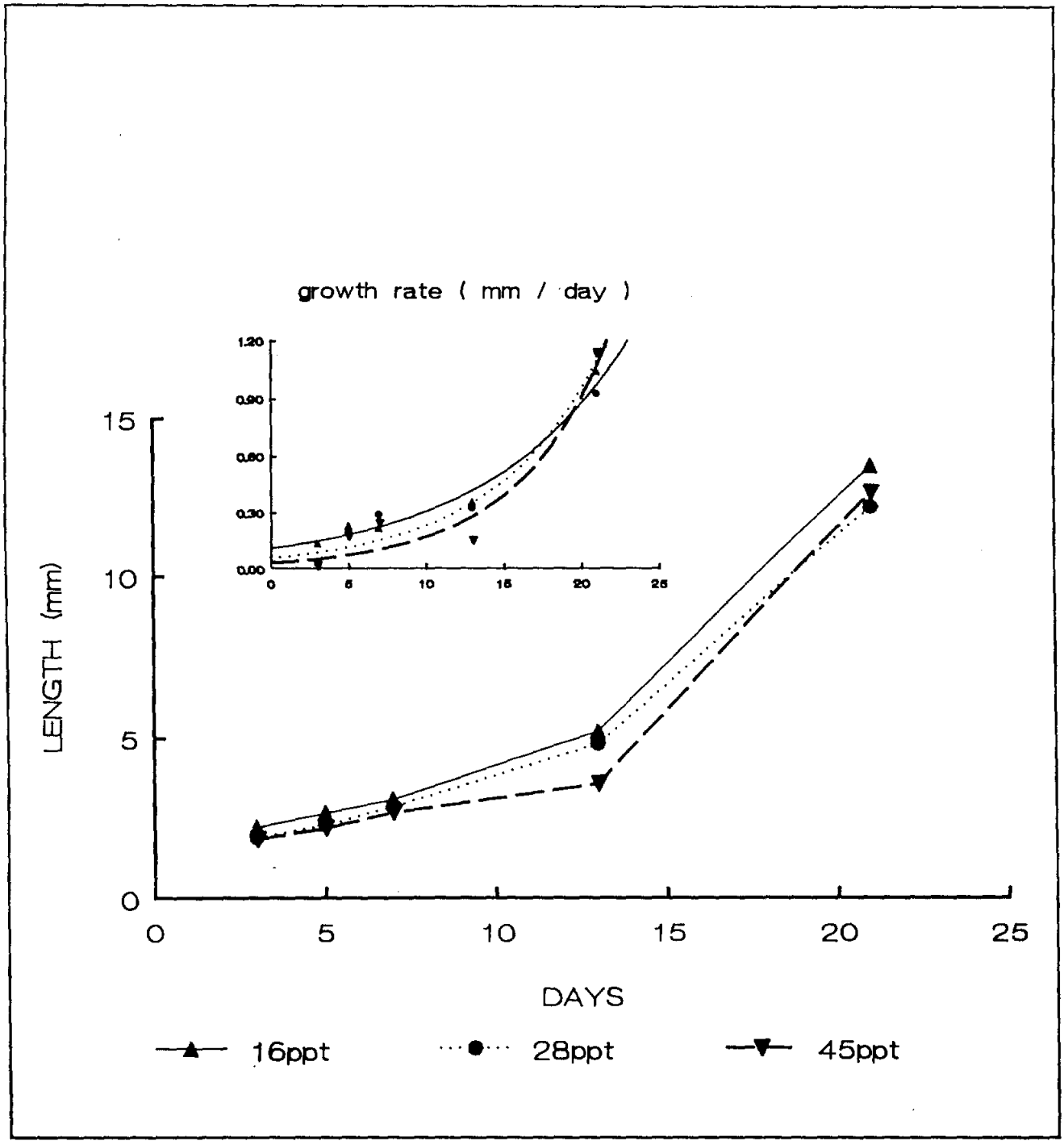

Figure 16. Growth data for spotted seatrout raised for three weeks in 16, 28 and 45 ppt salinity. Each point represents the mean size of larvae from three replicate tanks.

TABLE 11. Mean lengths and standard errors for spotted seatrout raised for three weeks in 16 ppt, 28 ppt and 45 ppt salinity. Means with same letter are not significantly different (Duncan's multiple range test).

AGES(DAY)	3	5	7	13	21
SALINITY					
16	2.20 ± .12^{A}	2.65 ± .24^{A}	3.08 ± .11^{A}	5.20 ± .18^{A}	13.55 ± .43^{A}
28	1.90 ± .06^{A}	2.28 ± .12^{A}	2.86 ± .12^{A}	4.80 ± .06^{B}	12.22 ± .15^{B}
45	1.85 ± .03^{A}	2.19 ± .09^{A}	2.68 ± .08^{A}	3.58 ± .14^{C}	12.70 ± .36^{B}

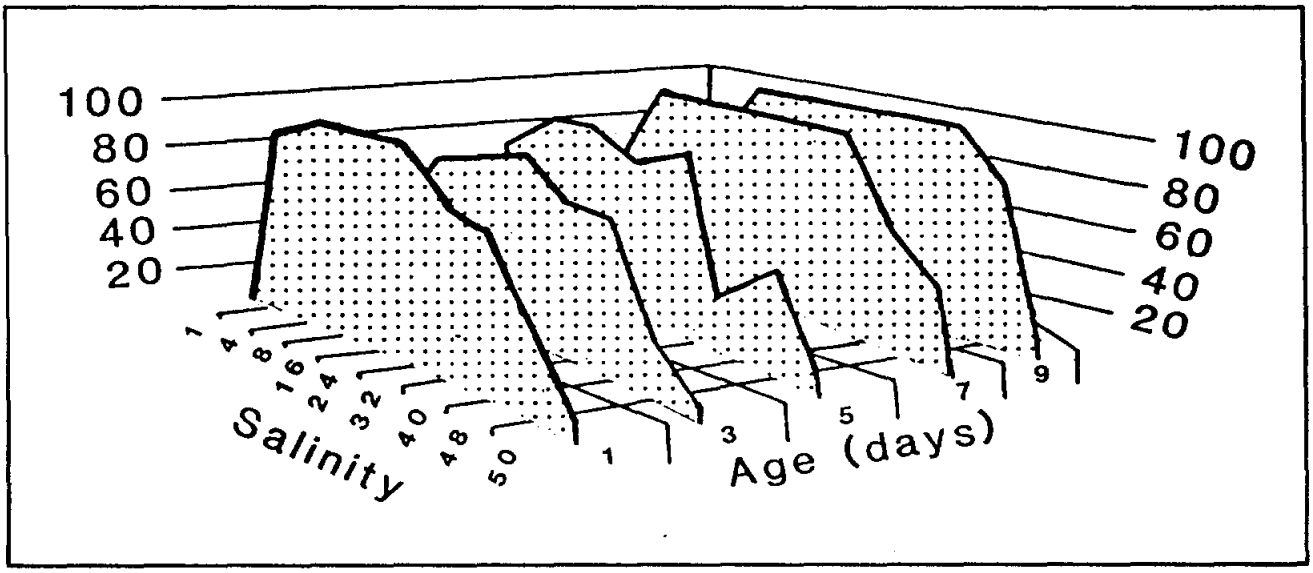

Figure 17. Percent survival for spotted seatrout larvae in eighteen hour salinity tolerance test (test range 0-50 ppt, spawning salinity 32 ppt, n = 3).

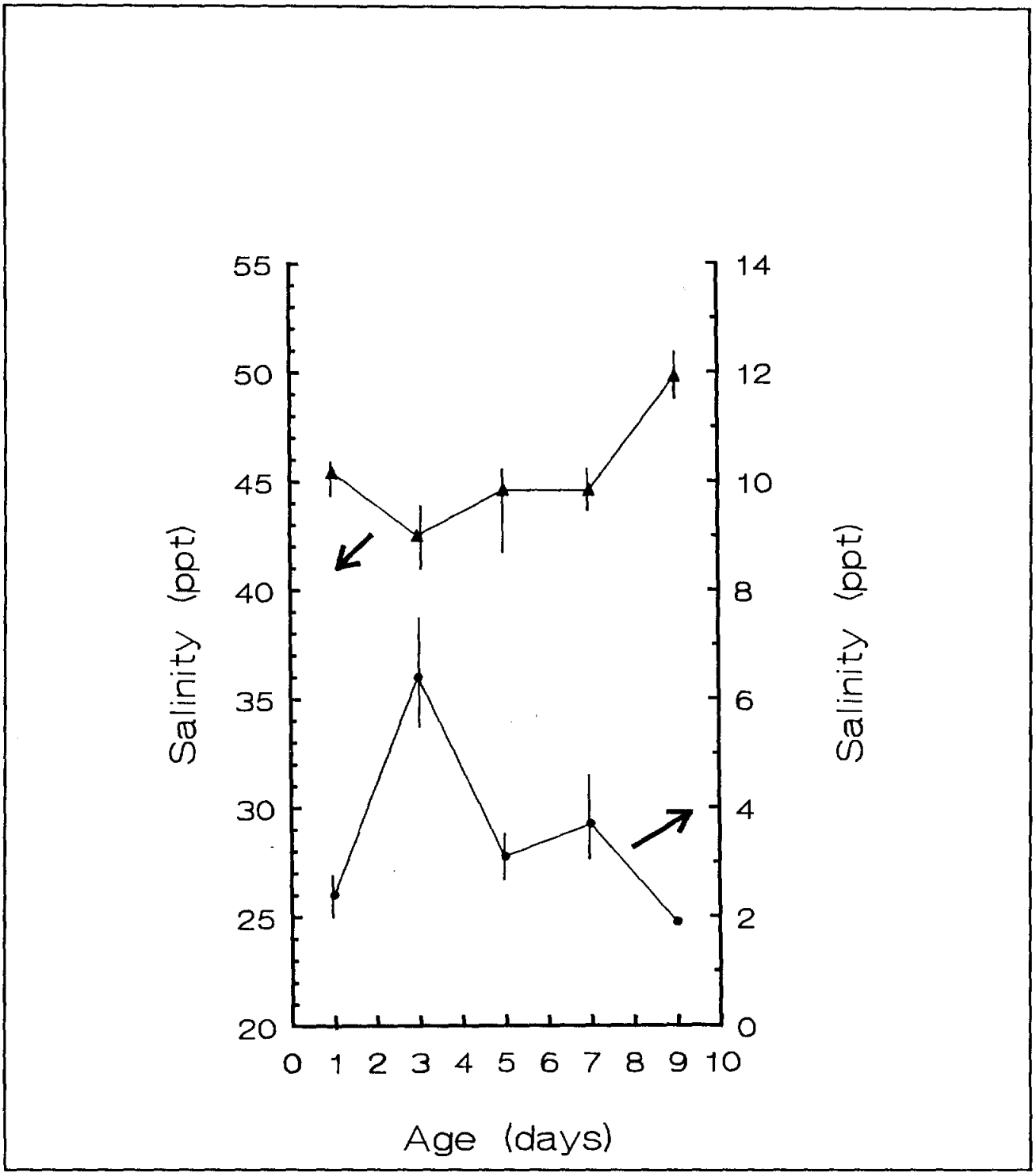

Figure 18. LD_{50} values for high and low salinity tolerance of spotted seatrout larvae reared at 32 ppt. Bars indicate upper and lower fiducial limits ($P \geq 0.95$).
Triangles - high salinity tolerance
Circles - low salinity tolerance

Survival results from similar acute salinity tolerance tests conducted for red drum and Atlantic croaker are presented in Tables 12 and 13.

LD_{50} values generated from regression analysis of these survival data are shown in Figures 19 and 20 for red drum and Atlantic croaker respectively. In comparison to spotted seatrout (Figure 18) it is clear that both red drum and Atlantic croaker have smaller ranges of tolerance. This comparatively broader range of tolerance for spotted seatrout is consistent with their more tolerant behavior demonstrated by early survival criteria in the hatch rate experiments described previously (Figures 13, 14 and 15). In contrast, juvenile and early adult red drum have been described as more euryhaline than Atlantic croaker and spotted seatrout by Wohlschlag (1981) following demonstration that red drum have more metabolic scope over a broader salinity range than spotted seatrout and Atlantic croaker.

These differences point to possible species specific differences in osmoregulation which become most apparent when larvae begin to feed (most obviously demonstrated at day three for spotted seatrout (Figure 18)). Differential ontogenic changes in the salinity tolerance in these species lead to their ability to invade euryhaline estuaries, either as eggs, advanced larvae or juveniles.

It is well accepted that an organism is a changing entity, its physiological state from day to day being continuously modified by its environmental history; thus acclimation is defined as "conditioning of the individual by its experience" (Fry, 1971).

Acclimation procedures are included in the spotted seatrout investigation to address these important considerations as well as to simulate events that are most likely to occur in the natural environment.

Table 12. Percent survival for red drum larvae in eighteen hour salinity tolerance tests (test range 0-50 ppt, spawning salinity 32 ppt, n = 3)

Treatment	Day 1	Day 3	Day 5	Day 7	Day 9
0	0.00	0.00	0.00	0.00	0.00
3	63.89	0.00	7.54	25.93	3.70
5	60.15	52.32	93.92	77.36	83.35
7	65.48	83.16	94.44	98.04	100.00
10	63.71	68.50	87.88	100.00	84.47
15	72.22	73.33	88.1	100.00	100.00
32	78.59	68.33	78.79	86.32	96.11
36	62.04	22.74	48.96	75.40	82.27
40	46.01	30.22	68.33	73.12	73.64
45	43.33	2.78	21.69	27.65	54.66
48	33.74	2.56	14.07	13.89	3.33
50	0.00	0.00	4.76	2.78	0.00

Table 13. Percent survival for Atlantic croaker larvae in eighteen hour salinity tolerance tests (test range 0-50 ppt, spawning salinity 32 ppt, n = 3)

Treatment	Day 1	Day 3	Day 5	Day 7
0	0.00	0.00	0.00	0.00
3	16.67	0.00	48.53	17.63
5	78.35	65.56	81.25	100.00
7	82.46	60.00	94.44	73.45
10	82.63	57.78	63.89	62.92
15	93.75	72.18	100.00	65.79
30	86.36	70.77	64.29	62.50
40	42.59	83.33	25.00	51.92
45	16.15	30.96	0.00	13.96
48	3.03	34.81	0.00	9.05
50	0.00	0.00	0.00	0.00

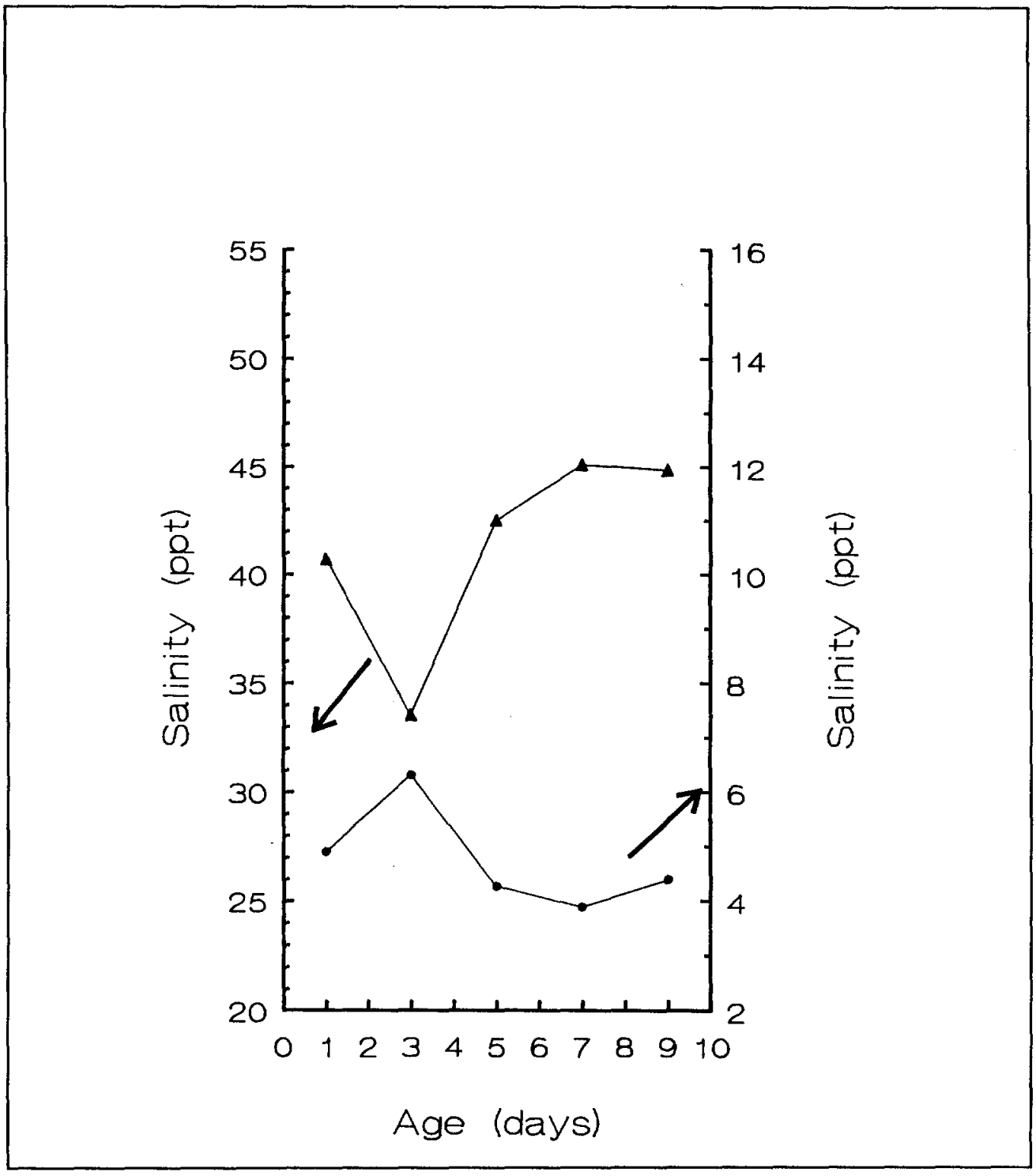

Figure 19. LD_{50} values for high and low salinity tolerance of red drum larvae reared at 32 ppt.
Triangles - high salinity tolerance
Circles - low salinity tolerance

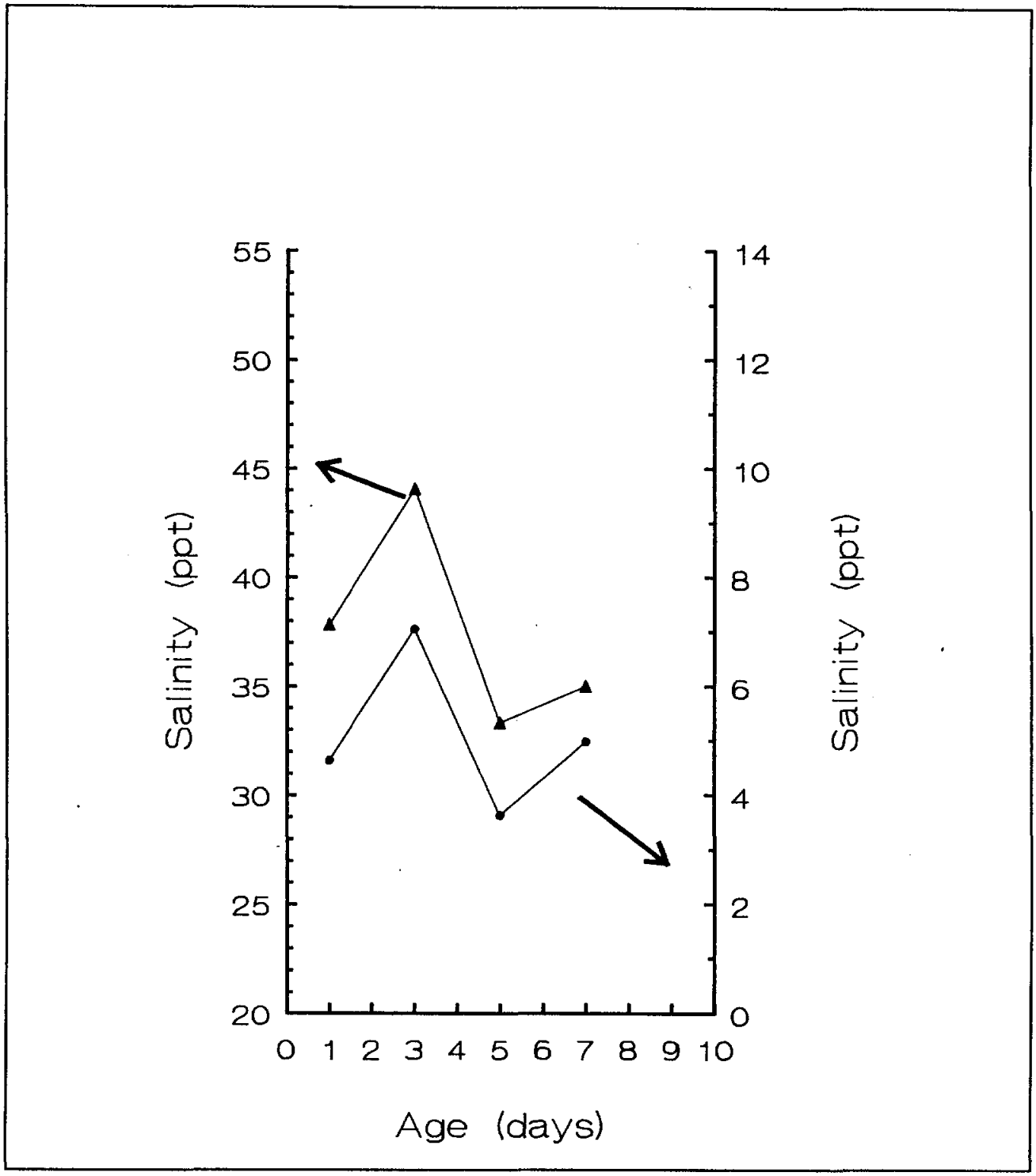

Figure 20. LD_{50} values for high and low salinity tolerance of Atlantic croaker reared at 32 ppt.
Triangles - high salinity tolerance
Circles - low salinity tolerance

Acclimation (20 ppt) to low saline conditions was demonstrated for seatrout larvae spawned in 32 ppt water by greater tolerance in 4 ppt by the acclimated larvae compared to the controls (Figure 21). Significant difference between the tolerance in these two groups of larvae was demonstrated ($P < 0.0001$). Conditions causing acclimation are likely to be present in natural situations, thus the lower limit in the natural situation may well show greater tolerance than that described in this investigation. In contrast, no significant hypersaline survival response could be demonstrated for acclimated larvae (40 ppt) compared to the control larvae (32 ppt), indicating that upper acclimation may not be possible under these conditions (Figure 22). The fact that the spawning salinity (32 ppt) was close to the upper limit (42.5 ppt) of the range tolerated for 50% survival, may be an important qualification. Perhaps maximum upper acclimation is already in effect at 32 ppt.

Acute salinity tolerance tests of spotted seatrout larvae from fish spawned in low salinity (24 ppt) were compared to offspring spawned in high salinity (32 ppt). Upper LD_{50} values were consistently lower for larvae from low salinity spawning (Figure 23). This raises some interesting questions. Firstly, at which developmental stages do acclimation experiences have important effects? Clearly there must be some maternal involvement. Although eggs spawned in 32 ppt water sink when placed in water of 20 ppt, viable pelagic eggs have been collected on the surface at 19 ppt during natural spawning times (Holt *et al* 1988). Larval acclimation demonstrated in this investigation indicate important effects resulting from the salinity in which spawning and early survival occurs. Alternatively, do these different tolerance criteria (from the two different saline spawning sites) represent results from two different populations, each with independent osmoregulatory needs and

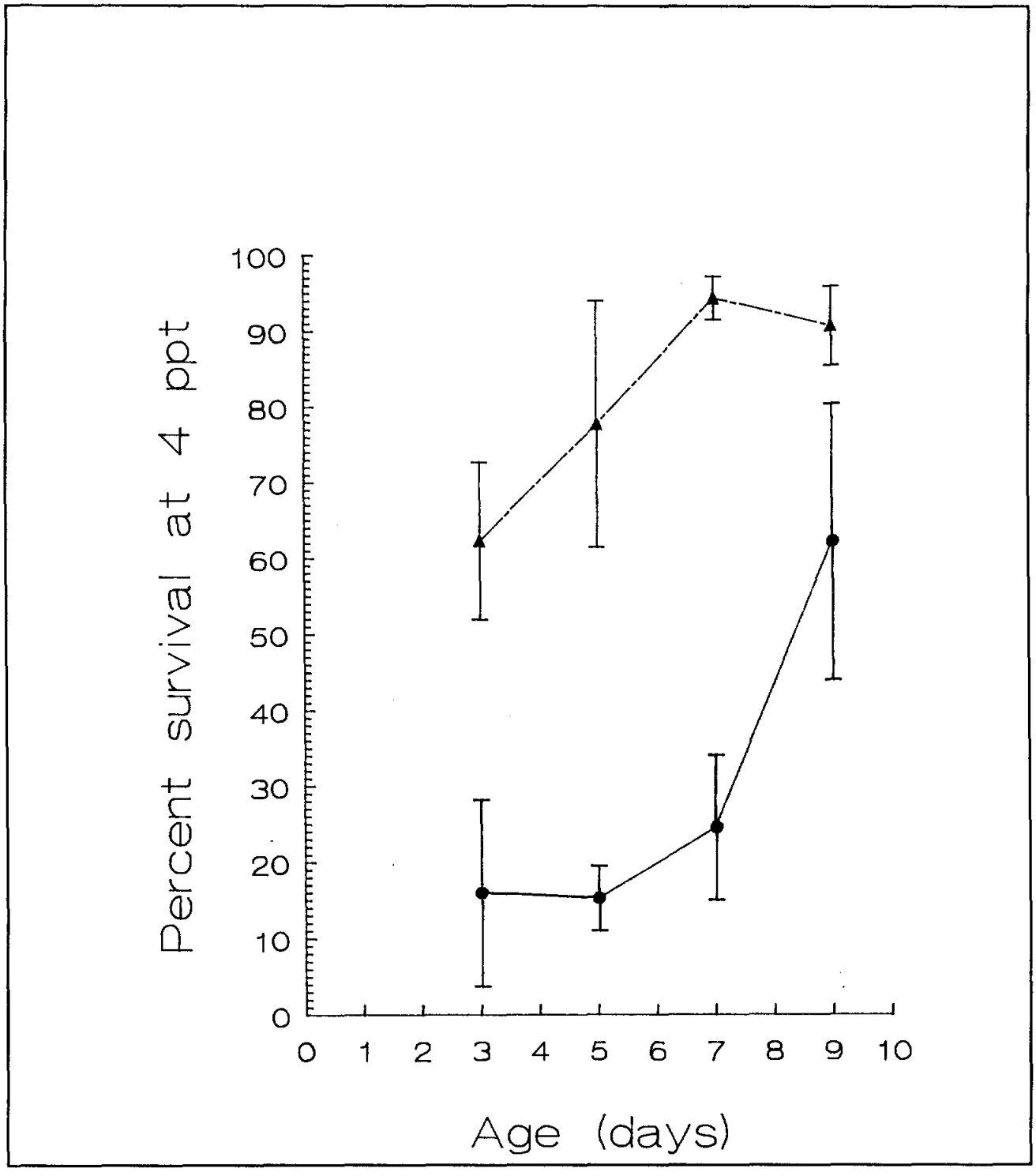

Figure 21. Percent survival for spotted seatrout larvae at a test salinity of 4 ppt. Bars indicate one standard error (n = 3).
Triangles - acclimation larvae (20 ppt rearing)
Circles - control larvae (32 ppt rearing)

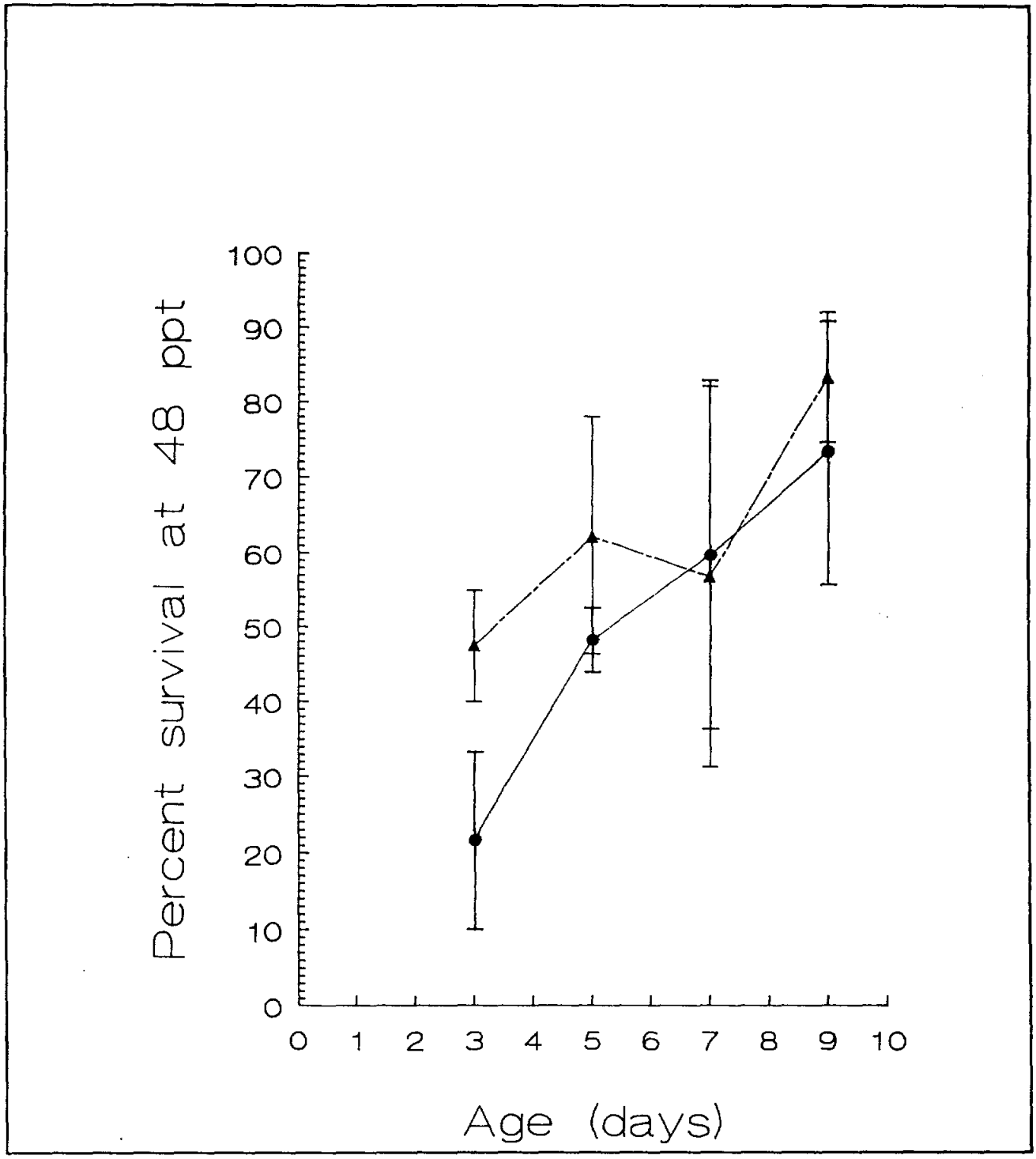

Figure 22. Percent survival for spotted seatrout larvae at a test salinity of 48 ppt. Bars indicate one standard error (n = 3).
Triangles - acclimation larvae (20 ppt rearing)
Circles - control larvae (32 ppt rearing)

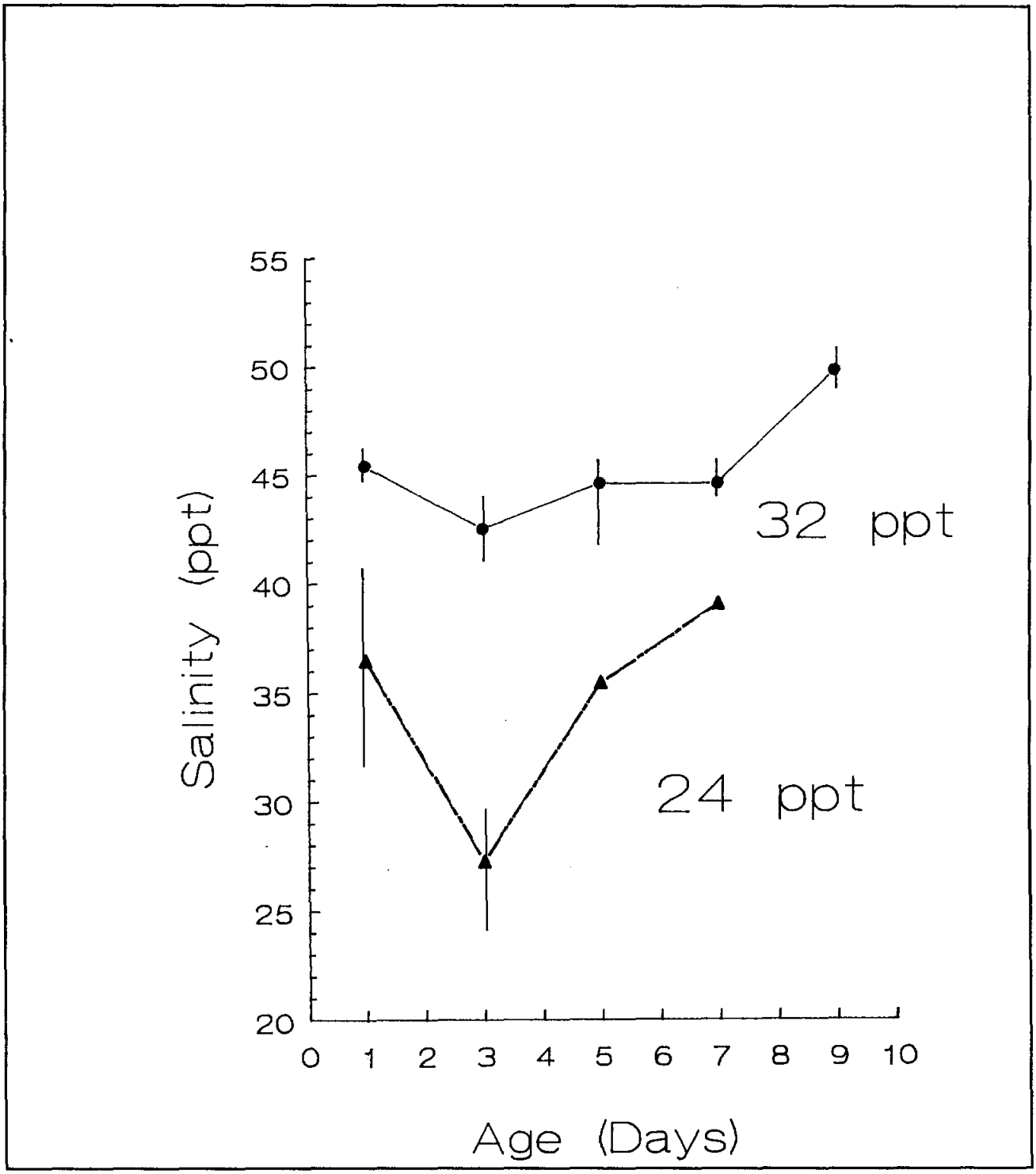

Figure 23. LD_{50} values for hypersaline tolerance of spotted seatrout larvae from different spawning salinities. Bars indicate upper and lower fiducial limits ($P \geq 0.95$).
Circles - Aransas Bay (spawning salinity of 32 ppt)
Triangles - Copano Bay larvae (spawning salinity of 24 ppt)

abilities? Weinstein and Yerger (1976) in an electrophoretic investigation of subpopulations of spotted seatrout from 6 estuaries in the Gulf of Mexico (including San Antonio Bays and Galveston Bay) and one on the Atlantic coast of Florida found that each estuary had discrete populations of seatrout, differences among the populations increasing with geographic distance. Although the spawning sites investigated in the present writing are in much closer proximity, studies addressing these questions should be very enlightening.

D. Integration

It is probable that a number of factors contribute to the survival response in tests such as those in this investigation. Natural mortality in larval stages of fishes is typically high (5-25% d^{-1}) (Dahlberg, 1979) with year to year variation in mortality rates during the larval period often cited as a major causal factor in the variability in year class strength (Leiby, 1984). Nevertheless, laboratory conditions allow the exercise of a certain amount of control over extraneous parameters, although control is limited and sometimes has complicating side effects. It is important to note that these LD_{50} results represent acute salinity tolerances determined under laboratory conditions while attempting to minimize the effects of non-salinity related parameters. Complicating factors such as feeding, swimming ability, prey detection and many others, not incorporated in this investigation but important in the natural environment, may well result in different real salinity tolerance in natural situations.

Variance inherent within any population renders end-points such as 100% survival and 100% mortality very difficult to quantify, whereas median lethal dose (LD_{50}) criteria (determined using probit analysis in this investigation) are statistically well defined (Finney, 1971). It is very difficult however, to extrapolate upper and lower saline limits within which

stable fisheries populations could be expected from the above determined LD_{50} values. Fifty percent survival due to one causal factor (salinity) is a perilous start in a multifaceted environment with numerous causal mechanisms affecting mortality. The acute salinity tolerance determined however, provides information valuable in determining patterns of changing tolerance and delimiting the range of extreme tolerance possible.

To address these important considerations, evaluation from regression analysis of the acute salinity tolerance criteria were made to determine the salinity range for each species in which there was negligible salinity related mortality (ie. mortality was little different to that in the controls). This information was integrated with hatch rate and 3^{-d} survival data to define salinity limits for the three species (Fig. 24). Again, the more euryhaline behavior of seatrout larvae in comparison to red drum and Atlantic croaker was apparent. The ranges presented in Figure 13 are probably far more applicable in accessing limits within which adequate larval recruitment and population survival could be relied upon. There is additional evidence that reinforces the lower salinity limits. Sperm motility of several sciaenids, including spotted seatrout and Atlantic croaker, is highest in fluids of 600 to 1000 mOsm (equivalent to 2/3 to full strength sea water) and declines linearly to zero motility at 300 mOsm (Gwo, Jim-Chywan, personal communication).

Field data provides important support for these tolerance limits. Rutherford *et al* (1986) describe successful larval catches in the Everglades National Park, Florida, only occurred within the salinity limits of 8-35 ppt (mean 25 ± 7.0 ppt) for red drum compared with a broader range of occurrence for spotted seatrout of 8-40 ppt (mean 33.2 ± 1.7 ppt). Lewis and Judy (1983) in documenting the occurrence of Atlantic croaker larvae in Onslow Bay and Newport River Estuary, North Carolina describe that only post-flexion larvae were

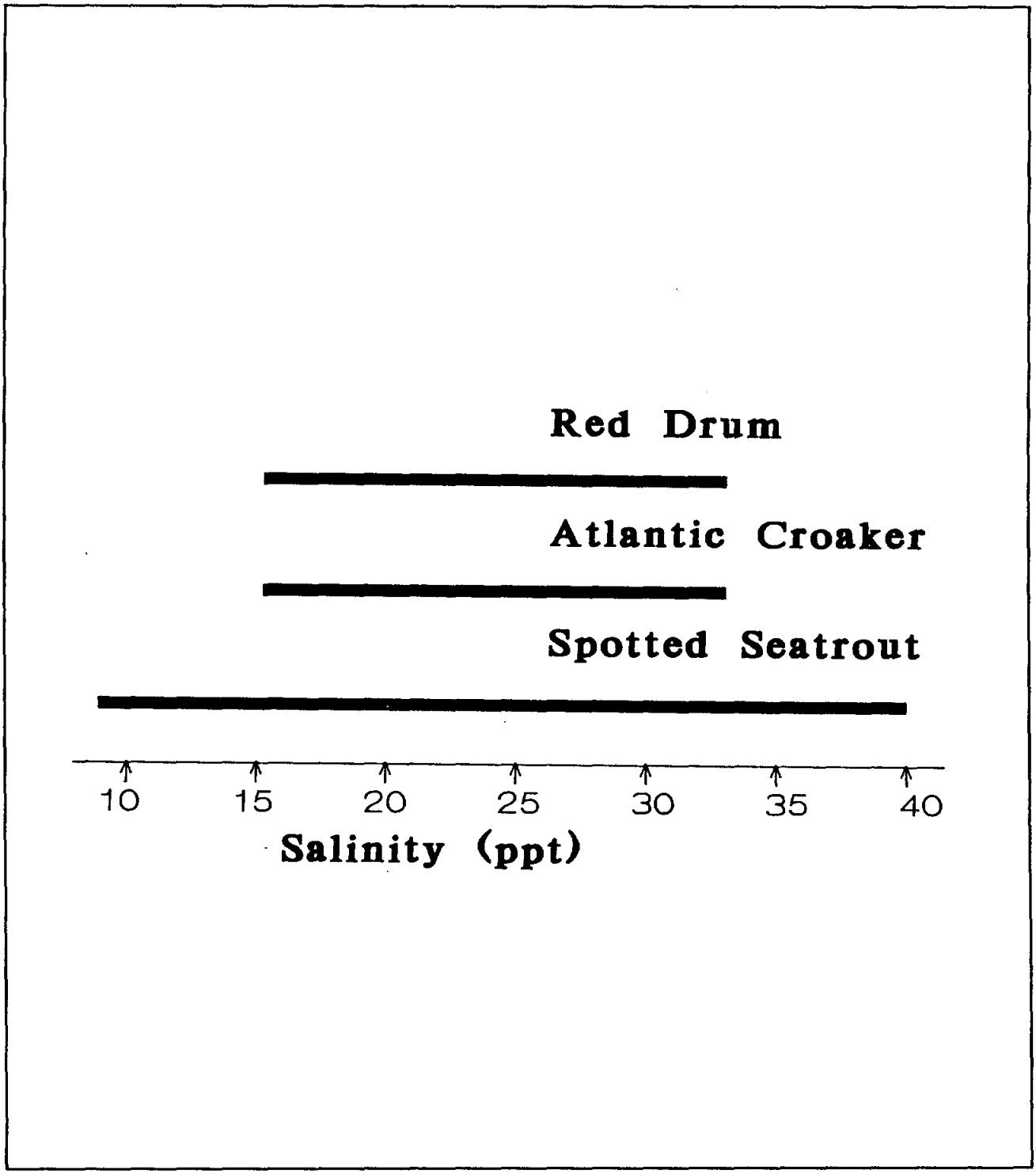

Figure 24. Salinity limits for no salinity related mortality during the pelagic larval stage of three species of sciaenids spawned in near full strength sea water and reared under optimum temperature conditions.

caught at estuarine stations with the earlier stages of development occurring further offshore. In south Texas bays, larval Atlantic croaker less than 10 mm are seldom taken in plankton tows except in Gulf passes in salinities of 20 ppt or greater, although larger Atlantic croaker (> 10 mm, post-flexion) are commonly collected in the upper bays in low salinity water (S. Holt and Arnold, TWDB Report, 1989). Thus the naturally occurring conditions documented for Atlantic croaker early larvae are most stable (open ocean salinity ± 35 ppt) in comparison to the other two species.

Spotted seatrout display broad salinity tolerance from hatching onward which is influenced by parental stock, or salinity of parental stock, and by acclimation. Smaller eggs collected from the high salinity sites do not merely reflect the spawning and fertilization salinity since some of the same eggs, experimentally fertilized in lower salinities (0, 10 and 20 ppt), were not different in size. It is apparent that egg size is determined before spawning, perhaps during final oocyte maturation. Hypersaline conditions such as those encountered in Laguna Madre and elsewhere along the Texas and Mexican coasts, may limit spawning in those areas during some part of the spawning season or some years. The presence of very large fish with partially spent ovaries in the Laguna Madre in August suggests spawning occurred there earlier in the season, perhaps when the salinity was lower.

Acclimation of spotted seatrout embryos increased their salinity tolerance. Alderdice (1988) suggested the significant effect of incubation salinity on salinity tolerance of larval Pacific herring *(Clupea pallasi)* could be due to a greater differentiation of regulatory tissue ie. chloride cells. Estuarine spawners such as spotted seatrout will be subjected to wide ranges in salinity during a long spawning season, and acclimation of the offspring, mediated in some way by the spawning adults, would be invaluable. Further acclimation of larvae

to changes in salinity (up or down) would ensure high survival under unpredictable conditions.

Red drum have a much more clearly defined, shorter period in which they spawn. In south Texas red drum spawn from mid-August through October in the near shore Gulf of Mexico (Holt *et al.*, 1988). In Florida waters red drum spawn during the yearly salinity minimum (23.5-32.4 ppt) according to Jannke (1971). Peters and McMichael (1987) reported spawning near the mouth of Tampa Bay from mid-August through November. They collected young red drum (≤ one-week old) only in the lower bay in 25-34 ppt salinity. Thus it seems reasonable that due to the more predictable nature of the salinity conditions in their spawning habitat that red drum need not have evolved as broad a salinity tolerance as spotted seatrout.

Similarly Atlantic croaker spawn well off shore in even more consistent saline conditions only invading the estuaries at later stages. Thus their lesser salinity tolerance range than spotted seatrout is well explained.

Temperature effects have not been investigated in this study, conditions being maintained to simulate optimum parameters. Fish are essentially thermal conformers with every response and every process proceeding within a thermal range dictated by the immediate environment (Brett, 1970). Thus interaction effects between temperature and salinity tolerance are very important considerations, especially in light of extreme conditions likely to occur in the natural environment. Gordon (1964) considered that tolerance to salinity change was greater at low than at high temperatures although the interaction is such that an optimum range for each species could be expected. Studies of combined effects of temperature and salinity generally show an optimum temperature at which eggs

are viable over a wide range of salinities, and reduced viability at high temperature, high salinity and low temperature, low salinity combinations (Alderdice and Forrester, 1971). Red drum eggs hatch poorly at low salinity, high temperature or low temperature combinations, and yolk-sac larval survival is reduced at high temperature, high salinity conditions (Holt *et al.*, 1981). Thus results reported herein may be modified by temperature extremes and species specific consideration of possible temperature related effects should be employed in final evaluation of salinity tolerance limits.

CONCLUSIONS

Salinity tolerance during larval development:

1) differed among the three species examined
2) was restricted during early egg development and at the end of the yolk-sac stage
3) extended further in the low salinity than in the high salinity range
4) increased with age after first feeding
5) in spotted seatrout was a function of parental salinity and acclimation and
6) had a range encompassing more extreme salinities for spotted seatrout compared to red drum and Atlantic croaker.

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