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Enhanced survival in striped bass fingerlings after maternal triiodothyronine treatment

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Abstract

Elevation of the triiodothyronine (T_3) content of striped bass (*Morone saxatilis*) eggs by maternal T_3 injection confirms the uptake of T_3 by oocytes. The resulting offspring were influenced favorably by the T_3, as seen in quantitative indices of development. As reported previously, larvae from T_3-supplemented eggs raised under laboratory conditions exhibited increased body area, length, dry weight, and rates of swimbladder inflation and survival, compared to controls. Also, the T_3 content of unfertilized oocytes correlated positively and highly significantly with survival to two weeks of age within individual cohorts (Brown *et al.*, 1988). In the present study, the survival of experimental and control striped bass was monitored through the fingerling stage, under hatchery production conditions. The rate of recovery of maternally T_3-treated cohorts from pond-culture was approximately fourfold that of controls. The striking effects of T_3 enrichment of eggs on offspring indicate the potential contribution of maternal hormones in striped bass development, and suggest possible applications in aquaculture.

Introduction

The sensitivity of larval and juvenile teleost fishes to exogenous thyroid hormones has been established for many years (see Pickford 1957), and a preponderance of beneficial effects of hormone treatments has been described (reviewed by Brown and Bern 1988). These include the promotion of growth, differentiation and survival rates, although some untoward side effects have also been seen. It is not clear to what degree the inconsistencies in the patterns of larval response to thyroid hormone exposure result from species differences and/or differences in the technical approach. For the most part, interest in the capacity of thyroid hormones to accelerate development and otherwise stimulate larval fish has been restricted to experimental endocrinology, despite the fact that the potential for applications in aquaculture is cited routinely. This may result from lingering doubts about the reliability of thyroid hormone supplements and the risk of undesirable complications (such as skeletal deformities: Higgs *et al.* 1979); also, the customary immersion treatment has inherent limitations and may be impractical in some culture systems.

Perspectives on the role of hormones in early development have been changed by observations that thyroid and probably other hormones are present in the yolk of all species of fish eggs in which they have been sought (Kobuke *et al.* 1986; Brown *et al.* 1987;

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Tagawa and Hirano, 1987; Greenblatt *et al.* 1988). If hormones of maternal origin influence early development, it follows that variations in maternal endocrine activity may modify the hormone profile in the eggs and perhaps alter subsequent development of offspring. This hypothesis was tested in a recent study in which gravid female striped bass (*Morone saxatilis*) were injected with triiodothyronine (T_3) shortly before spawning, and the effects on eggs and larvae were quantified (Brown *et al.* 1988). The injections caused elevations in egg T_3 content which correlated closely with survival through the first two weeks after hatching, and which were consistently associated with improved rates of swimbladder inflation. In the current study, we have monitored the survival of striped bass from maternally T_3-injected and vehicle-injected females over a longer period of time, focussing on the recovery of fingerlings from pond-culture, reared under hatchery production conditions.

Materials and methods

Female striped bass (*Morone saxatilis*) were collected in the Sacramento River by the California Department of Fish and Game by electroshocking during the 1987 striped bass spawning season. Eight gravid females showing ripe follicles after microscopic examination of eggs sampled by catheter, were selected. All eight were injected with human chorionic gonadotropin (HCG; 130 IU per pound body weight) within a few minutes after capture. Four of the fish were also injected with T_3 (sodium salt, Sigma, in dimethylsulfoxide (DMSO) at a concentration of 200 to 250 mg/ml). The dosage of the intramuscular hormone injections was 20 $\pm$ 2 μg hormone/g body weight (after Detlaff and Davydova 1979). The remaining four prespawning female striped bass were injected with DMSO alone.

These eight female and approximately 20 uninjected male striped bass were transported to the Central Valley Hatchery in Elk Grove CA, where they were spawned (usually about 48 hours after capture). Eggs were handstripped from each female, fertilized with the milt from two or three males, and then transferred to McDonald jars for hatching. The same eight female striped bass were used for this report as in the study by Brown *et al.* (1988), but the larvae were separated at the time of hatching and were reared independently in the two studies. In this study, newly-hatched larvae were moved to aerated glass aquaria where they were reared at a temperature of approximately 16–20°C. The salinity was increased gradually, from 1.75‰ for the firth 24 hours to 3.3‰ beginning at 96 hours, using Instant Ocean salts. Following yolk-sac absorption, the larvae were stocked into 0.3-acre ponds. Initial stocking densities ranged from 93,500 to 187,000 per acre, depending on the availability of ponds and larvae at any given time. Eleven ponds were stocked exclusively with fry from females injected with T_3 at the specified dosage, and six ponds were stocked exclusively with the fry produced by the vehicle-injected females. The mean initial stocking densities did not differ significantly between the treated and control groups (Table 1). The diet in the ponds consisted of assorted zooplankton, enhanced by application of fertilizers (cottonseed meal, alfalfa pellets, and liquid organic fertilizers). Water chemistry in the ponds was monitored twice each week, and maintained within limits (pH, 7.2–9.0; temperature, below 22°C; dissolved oxygen, above 4 ppm) by adding fresh well-water and by judicious use of the fertilizers. The striped bass were harvested from the ponds approximately one month after stocking, and the number of fingerlings surviving in each pond was estimated. Mann-Whitney tests were used to compare survival and yield per acre data from the two treatment groups. Two of the experimental ponds suffered complete mortality due to algal blooms and subsequent anoxia; these data were not included in our comparison. After the ponds were harvested, fingerlings were transferred to throughs where they were continually sorted by size and eventually placed in raceways. Unfortunately, it was not possible to maintain separate treated and untreated stocks throughout this grading process, so we were unable to examine the effects of the maternal T_3 supplements beyond the pond-culture stage.

Table 1. Pond stocking rates and returns in T_3-treated and control (C) cohorts.

Treatment	Pond	Stocking density (fish per acre)	Density at harvest (fish per acre)	% Survival
T_3	1	187,500	25,000	13.3
T_3	2	176,364	12,063	6.8
T_3	3	93,548	41,465	44.3
T_3	4	131,818	45,811	34.8
T_3	5	176,910	21,867	12.4
T_3	6	124,490	29,896	24.0
T_3	7	122,449	12,322	10.1
T_3	8	125,091	28,707	22.9
T_3	9	109,091	34,576	31.7
T_3 group mean		138,251	27,969	22.3
(± SEM)		(11,216)	(3,894)	(4.2)
C	1	153,125	7,972	5.2
C	2	149,968	5,616	3.8
C	3	131,356	849	0.6
C	4	124,297	3,594	2.9
C	5	125,520	10,277	8.2
C	6	111,733	15,913	14.2
C group mean		132,619	7,363	5.8
(± SEM)		(6,504)	(2,176)	(2.0)

Results

The mean harvest from the ponds stocked from maternally T_3-treated progenies was approximately fourfold that of the control groups. These data were analyzed in terms of both the percentage of stocked larvae surviving to harvest and of the yield of fingerlings per acre (Table 1). Mann-Whitney tests indicated that the ponds stocked from the maternally T_3-treated females exhibited highly significantly greater percentage of survival ($p < 0.01$) and enhanced yield of fingerlings per acre ($p < 0.01$; Fig. 1).

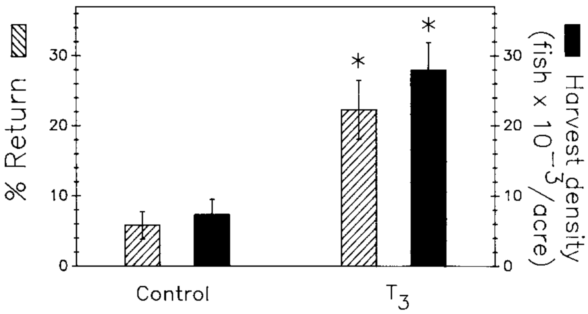

Fig. 1. Percentage of survival (hatched bars) and density of fish harvested (solid bars) from production ponds stocked with larvae from T_3-injected female broodstock (T_3) as compared with survival and harvest density data from ponds stocked with vehicle (DMSO)-injected controls (Control). Asterisks indicate significant differences between hormone-treated and control groups at the $p < 0.01$ level. N = 9 ponds (hormone-treated) and 6 ponds (control).

Discussion

The results of the previous study (Brown *et al.* 1988) revealed that the larvae from T_3-injected female striped bass raised in experimental containers showed consistent improvements in early survival over controls. The present results indicate that the advantages conveyed by yolk T_3 supplementation are not confined to the period of yolk-sac absorption, and that the prospects for long-term survival in ponds are also improved in these fish. We do not yet know the biological basis for the enhancement of fingerling survival by maternal T_3 injection, but several possible explanations seem plausible. If thyroid hormones promote differentiation of the central nervous system in fish as in mammals (reviewed by Shambaugh 1986), then numerous long-term benefits of early hormone exposure would be anticipated. It has also been proposed that some forms of dietary and environmental flexibility may require thyroid hormones (Specker 1988); again, an early advantage might be manifested in long-term benefits. Clearly, some of the differences in survival rate can be attributed to the previously observed stimulatory effect of T_3 on swimbladder inflation. The failure of the swimbladder to inflate seriously reduces the ability of fry to feed (Doroshov *et al.* 1981) and makes them more susceptible to cannibalism. We should point out, however, that even if we assume 100% mortality among the larvae that failed to inflate the swimbladder (Brown *et al.* 1988), this alone does not account for the differences in fingerling survival observed here.

The striped bass is not an ideal experimental animal for this sort of study. A limited number of gravid females has become available for research, and the space requirements of both the broodstock fish and the offspring are considerable. Because of the limited number of experimental fish, we were unable to run a set of uninjected (as opposed to vehicle-injected) controls as desired. However, the survival rate among the larvae and fingerlings produced by DMSO-injected females was comparable to that of uninjected striped bass reared under normal culture conditions, suggesting that any possible deleterious effect of DMSO did not affect the offspring. The reasons for the use of this particular solvent, the need for further comparison with uninjected controls, and concerns over the high hormone dosage have been discussed by Brown *et al.* (1988). Also, the culture of striped bass in ponds under hatchery conditions cannot be considered to be strictly controlled. For this reason, we wish to stress that confirmation and elaboration of our present results under more precisely uniform rearing conditions are desirable. On the other hand, there is no doubt that the performance of the treated cohorts was distinctly improved, regardless of the past history of the individual ponds in which the fingerlings were reared. In several cases, better-than-average survival rates and yields were obtained in ponds that have produced poorly over the years. Subjectively, hatchery employees have also reported that the larvae from T_3-treated females consistently display improved hardiness (appreciably better resistance to weather changes, handling, etc.).

It is clear from this and other recent studies that there is ample reason to continue to explore possible applications of thyroid hormones in fish culture. Certainly, any technique that can potentially multiply hatchery output (or, accordingly, reduce the labor and expense required to maintain a given level of production) requires further attention. In addition to the practical issues raised in these studies, numerous compelling scientific questions have arisen. At present, we are limited to speculation as to how a maternal infusion of thyroid hormone can increase fingerling survival. Virtually none of the basic research that has been done on the hormonal stimulation of early development in fish has probed more deeply than the organismal level. It is not known whether this sort of experimental hormonal boost of early development represents an acceleration of processes that normally occur at a slower rate, or whether such hormone supplements may be correcting for some deficiency (e.g., hypothyroidism). Such deficiencies, in turn, may be caused by a variety of factors, for example, physiological condition of wild broodstock in the San Francisco Bay, capture stress, and prematurely induced ovulation. In addition, the beneficial effect of thyroid therapy by injection of preovulatory female striped bass may involve routes other than yolk enrichment, such as improvement of ovulation by way of enhanced sensitivity of the ovary to gonadotropin (as shown in sturgeon by Detlaff and Davydova (1979)). We are only beginning to contemplate the possible implications of the deposition of thyroid hormones and other bioactive compounds, including substances that may have negative effects, from the maternal circulation into egg yolk.

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