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THE INFLUENCE OF TRIPLOIDY AND HEAT AND HYDROSTATIC PRESSURE SHOCKS ON THE GROWTH AND REPRODUCTIVE DEVELOPMENT OF PERCH (*Perca flavescens*) REARED TO ADULT SIZE UNDER SELECTED ENVIRONMENTAL CONDITIONS

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Abstract

We determined the influence of triploidy and shocks used to alter ploidy on the growth and reproductive development of adult perch reared under ambient and constant (near optimal) environmental conditions. These conditions assimilate pond and recirculation system aquaculture, respectively. Separate groups of perch eggs were treated with heat shocks (28-30 °C) for durations of 10-25 min, beginning at 2-5 min post-fertilization) or hydrostatic pressure shocks (9000 or 11 000 psi for durations of 8 or 12 min, beginning at 5 min post-fertilization) to induce triploidy in 30-70% of the eggs. After hatch, perch fry exposed to heat shock, pressure shock, or no shock were stocked and reared in separate ponds until they reached 25-35 mm total length (TL). The perch were harvested from the ponds and habituated to formulated feeds in separate tanks. When the fish reached 75-100 mm TL, ploidy was determined on individual fish by flow cytometry. Subsequently, the five groups of fish (unshocked diploids, heat-shocked diploids, pressure-shocked diploids, heat-shocked triploids, and pressure-shocked triploids) were stocked separately into two sets of five tanks each. One set of tanks was kept under environmental conditions that are near optimal for perch growth (21°C, 16h light/8h dark photoperiod), and the other set was kept under ambient conditions. The fish were then reared for 388 days. Because there were no apparent differences between heat-shocked and pressure-shocked fish, these groups were pooled for statistical purposes, and for each environmental condition the data were analyzed as a 2 x 3 factorial experiment, with the factors being sex (male or female) and treatment (unshocked diploid, shocked triploid, and shocked diploid). Under both environmental conditions, females grew much faster than males, and the shocks used to alter ploidy had a negative effect on growth that was independent of ploidy status. Under ambient (but not constant) environmental conditions, triploid perch grew faster than shocked diploids, and had higher fillet yields than either diploid group. Under both conditions, males had greater fillet yields than females. In general, the fillet yields of different groups of perch were inversely proportional to GSIs. Under both environmental conditions, female triploids had lower estradiol-

17β (E_2) levels than diploids. Taken together, these findings show that triploidy offers potential improvements in production characteristics of perch only if the negative effects of shocks can be avoided, and only to perch reared under ambient environmental conditions (e.g., pond culture). Our findings also show that the negative effects of shocks applied to newly fertilized eggs are permanent. In addition, the reduced levels of E_2 (which promotes perch growth) in triploid female perch may offset any increased growth potential associated with sterility.

Introduction

The yellow perch (*Perca flavescens*) is a highly valued food fish with numerous biological characteristics that make it an excellent candidate for commercial aquaculture (Calbert, 1975). Perch readily accept formulated feeds, show little aggressive or cannibalistic behavior, and are highly tolerant of intensive culture conditions. Expansion of the perch aquaculture industry, however, has been constrained by several growth and maturational characteristics of this species. First, the overall growth potential of perch is limited by its inherent small size and slow growth rate (Huh, 1975; Malison et al., 1985). Second, a considerable reduction in growth rate occurs well before perch attains a market size of 140-160 g (Huh, 1975; Schott, 1980; Malison et al., 1985). Third, gonadal development can decrease fillet yields (i.e., the percentage of edible flesh) in perch by up to 35% (Le Cren, 1951; Malison et al., 1986).

These three problems may all be associated with sexual maturation and gonadal development, which begin in perch during the first year of life (Malison et al., 1985; 1988a). It is widely believed that sexual development acts to channel energy into the production of eggs and sperm, thereby slowing somatic growth (e.g., Purdom, 1976; Utter et al., 1983). Accordingly, the induction of sterility in fish by induction of triploidy may enhance growth and increase the percentage of edible flesh.

We have previously demonstrated that heat and hydrostatic pressure shocks applied to fertilized eggs to induce triploidy exert a negative effect on the growth of juvenile perch that is independent of ploidy status (Malison et al., 1993b). Juvenile heat-shocked triploid perch showed retarded gonadal development in both sexes, and showed faster growth than heat-shocked diploids. The effects of similar ploidy manipulations on perch reared to adult size have not yet been reported. This study investigated such effects on perch reared under ambient and constant environmental conditions to assimilate pond and recirculation system aquaculture, respectively.

Materials and Methods

General Procedures

Experiments were conducted at our wet laboratory facilities located at Lake Mills State Fish Hatchery, Lake Mills, WI. All fish used were the offspring of wild brood fish collected from Lakes Mendota and Cherokee, Dane County, WI. Eggs were stripped from ripe females and fertilized using the dry method described by Heidinger and Kayes (1986). Triploidy was induced by exposing eggs to heat shocks of 28-30 °C for a duration of 10-25 min, beginning at 2-5 min post-fertilization, or hydrostatic pressure shocks of 9000 or 11000 psi for a duration of 8 or 12 min, beginning at 5 min post-fertilization (Malison et al., 1993a). The eggs were then incubated under a gradually increasing temperature regime (± 0.5 °C per day).

Experimental Design

On day 6 or 7 postfertilization, 10-20 embryos were randomly selected from each treatment group and their ploidy levels determined by flow cytometry as previously described (Malison et al., 1993a). Batches of embryos containing 30-70% triploids, and respective unshocked controls, were incubated until hatch and stocked into three separate production ponds (one for heat-shocked eggs, one for pressure shocked eggs, and one for unshocked eggs) and reared for approximately 40 days (to 25-40 mm total length [TL]). The fingerlings were then harvested and transferred into the laboratory, stocked into separate 750-l flow-through fiberglass tanks, and habituated to intensive culture conditions (Malison and Held, 1992) and formulated feed (Silver Cup salmon feed, Murray Elevators, Murray, UT or W-16, Glencoe Mills, Glencoe, MN).

Prior to the initiation of the growth trial, pit tags were implanted into the body cavity of the individual fish, and 3-6 ml of blood were collected from the pseudobranch artery for ploidy determination by flow cytometry. Shocked triploids were then separated from shocked diploids and the few mosaics detected (<1% of the fish) were eliminated from our studies. Unshocked control perch were treated in a manner identical to that described for shocked fish.

The five groups of fish (unshocked diploids, heat-shocked diploids, pressure-shocked diploids, heat-shocked triploids, and pressure-shocked triploids) were stocked separately into two sets of five 220-l flow through tanks each. One set of tanks was kept under ambient conditions, and these tanks received 4-6 l/min of unheated water that varied in temperature on a seasonal basis from 4-21°C, and had tank lighting that mimicked natural daylengths. The other set of tanks was kept under constant conditions, and these tanks were supplied with 4-6 l/min of tempered water at 21.0 ± 0.5°C, and lighting was set at 16 h light: 8 h dark photoperiod. Fish were hand-fed to satiation (about 2-5% body weight/day) depending on water temperature. Tanks were cleaned to remove excess food and feces once weekly. Fish were individually weighed and measured at the following sampling times: days 0, 17, 72, 140, 207, 275, 338, and 388. Condition factor (K) was calculated according to Carlander (1977): $K = [\text{weight (g)}/\text{standard length (mm)}^3] \times 10^5$

At the end of the experiment, five fish were randomly selected from each treatment group, killed with an overdose of MS-222, and blood samples were collected from the caudal vasculature for analyses of serum E_2 and testosterone levels. The hormones were measured using commercially available radioimmunoassay kits validated for use in perch (Diagnostic products, Inc., Los Angeles, CA). The gonads were weighed and gonadosomatic indices (GSIs) were calculated using the formula: GSI = [gonad weight (g)/body weight (g)] x 100. The fish were filleted and fillet yields were similarly expressed as percentage of body weight.

Statistics

For both environmental conditions there were no apparent differences between heat-shocked and pressure-shocked fish. Accordingly, these groups were pooled for statistical purposes, and for each environmental condition the data were analyzed as a 2 x 3 factorial experiment, with the factors being sex (male or female) and treatment (unshocked diploid, shocked triploid, and shocked diploid). The data were analyzed using analysis of variance followed by pre-planned orthogonal contrasts at P=0.05. Data expressed as percentages were evaluated using arcsine transformations as suggested by Sokal and Rohlf, 1995). All results were expressed as mean ± standard error of the mean (SEM).

Results

Ambient conditions

Data on weight gains showed that (1) females had higher weight gains than males, (2) among females, unshocked diploids had higher weight gains than the shocked groups, and (3) among males, triploids had higher weight gains than shocked diploids (significance of "sex", and "sex x ploidy ", see Table 1 and Figure 1).

Data on length gains showed that (1) females had higher length gains than males, (2) unshocked diploids had higher length gains than the shocked groups, and (3) triploids had higher length gains than shocked diploids (significance of "sex" and "ploidy", see Table 1 and Figure 1).

Data on condition factors showed that (1) females had higher condition factors than males, and (2) shocked diploids had higher condition factors than triploids (significance of "sex" and "ploidy", see Table 1 and Figure 1).

Data on fillet yields showed that (1) males had higher fillet yields than females, (2) shocked groups had higher fillet yields than unshocked diploids, and (3) and triploids had higher fillet yields than diploids (significance of "sex" and "ploidy", see Table 1 and Figure 1).

Table 1. Results of ANOVAs for various parameters of fish reared under ambient and constant environmental conditions.

Source of Variation	Weight Gain (g)	Length Gain (mm TL)[1]	Condition Factor (K)[2]	Fillet Yield (%)[3]	GSI (%)[4]	T (ng/ml)[5]	E_2 (ng/ml)[6]
Ambient Environment							
Sex	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01
Ploidy	ns	P<0.01	P<0.05	P<0.01	P<0.01	ns	P<0.01
2nSh vs 3n[7]	ns	P<0.01	P<0.01	P<0.01	P<0.01	ns	P<0.01
2nUn vs Sh[8]	ns	P<0.01	ns	P<0.01	P<0.01	ns	ns
Sex x Ploidy	P<0.01	ns	P<0.05	ns	ns	ns	P<0.01
2nSh vs 3n	P<0.05	ns	P<0.05	ns	ns	ns	P<0.01
2nUn vs Sh	P<0.05	P<0.05	ns	ns	ns	ns	ns
Constant Environment							
Sex	P<0.01	P<0.01	P<0.01	P<0.05	P<0.01	ns	P<0.01
Ploidy	P<0.01	P<0.01	P<0.01	ns	P<0.05	ns	P<0.01
2nSh vs 3n	ns	ns	ns	ns	P<0.05	P<0.05	P<0.01
2nUn vs Sh	P<0.01	P<0.01	P<0.01	ns	ns	ns	ns
Sex x Ploidy	ns	ns	ns	ns	P<0.05	ns	P<0.01
2nSh vs 3n	ns	ns	ns	ns	P<0.05	ns	P<0.01
2nUn vs Sh	ns	ns	ns	ns	ns	ns	ns

[1] TL = total length; [2] K = (body weight (g)/standard length [mm^3] x 10^5; [3] Fillet yield = (fillet weight [g]/body weight [g] x 100;[4] GSI = gonad weight (g)/body weight (g) x 100; [5] T= testosterone; [6] E_2= estradiol-17β; [7] 2nSH= shocked diploids, 3n= triploids;[8] 2nUn= unshocked diploids, Sh= shocked diploids + triploids

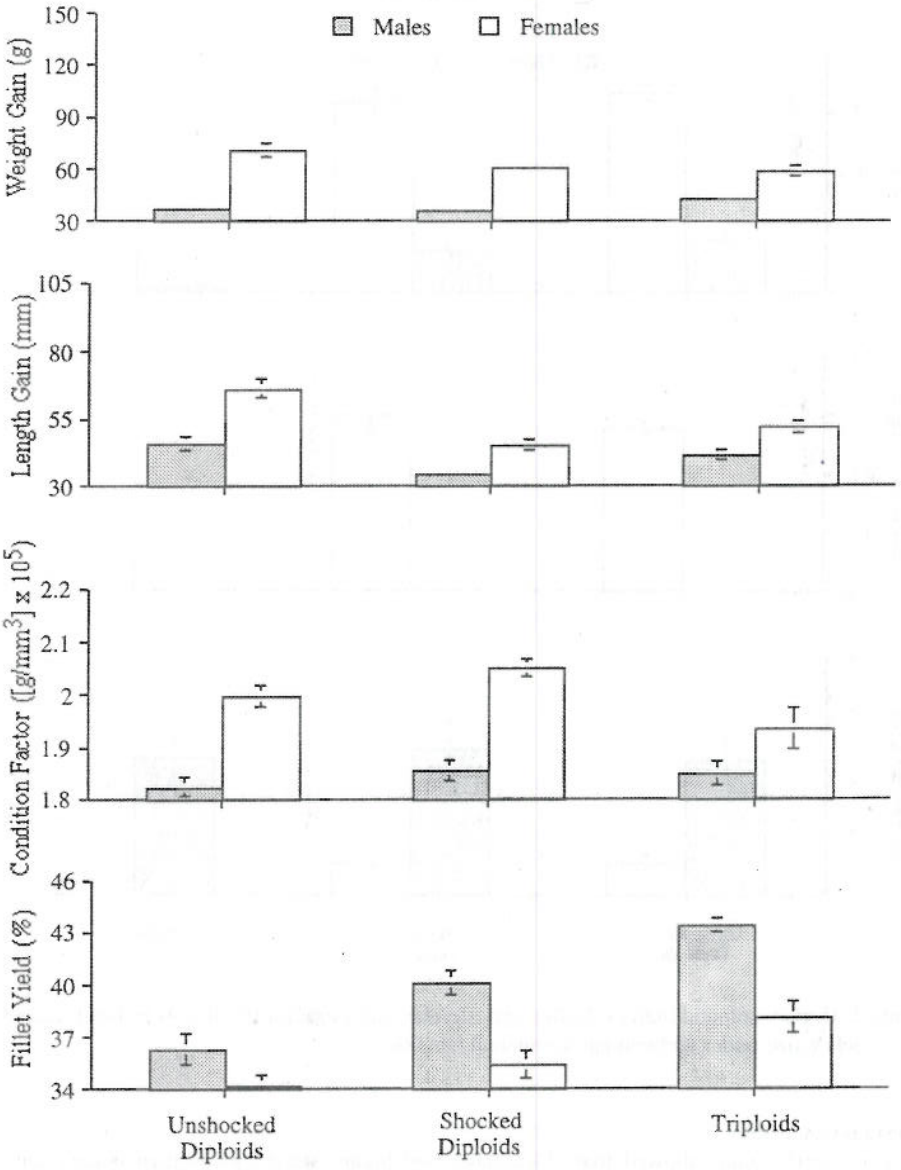

Figure 1. Weight gains, length gains, condition factors, and fillet yields in perch reared to adult size under ambient environmental conditions.

Data on GSIs showed that (1) females had higher GSIs than males, and (2) shocked diploids had higher GSIs than triploids (significance of "sex" and "ploidy", see Table 1 and Figure 2).

Testosterone levels were higher in males than in females. Data on E_2 levels showed that (1) females had higher levels than males, and (2) in females, diploids had higher E_2 levels than triploids (significance of "sex", "ploidy", and "sex x ploidy", see Table 1 and Figure 2).

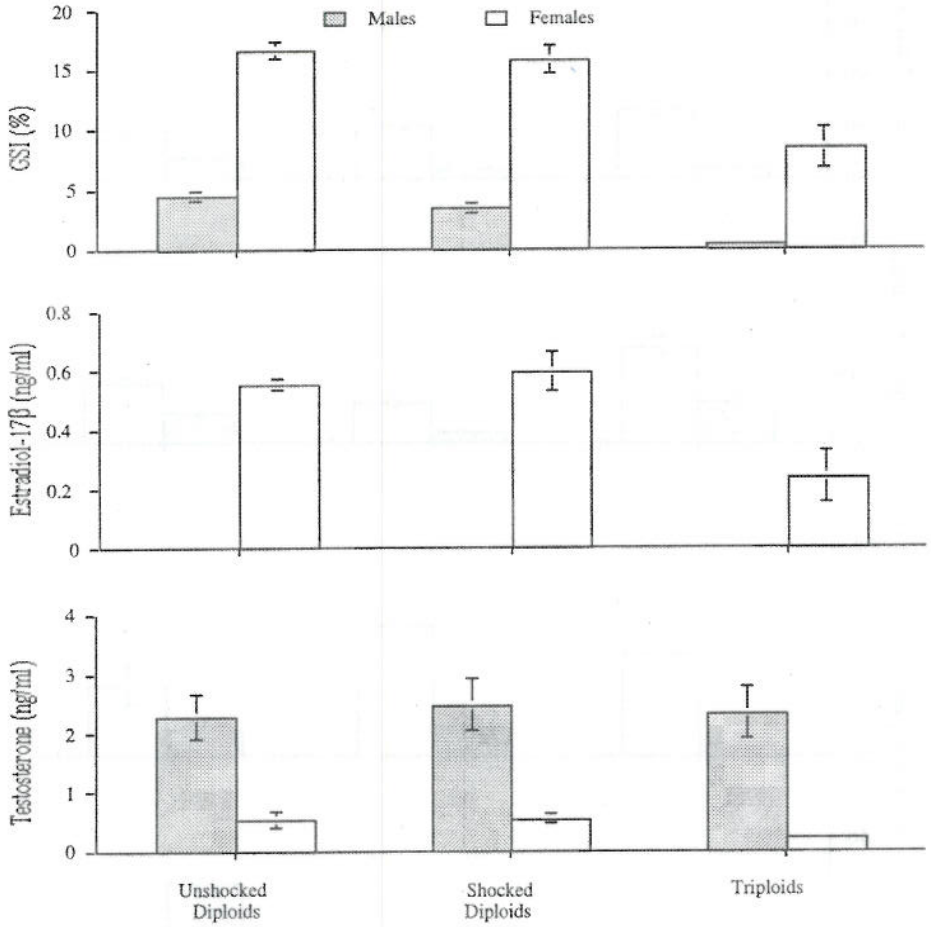

Figure 2. Gonadosomatic indices (GSIs), testosterone, and estradiol (E_2) levels in perch reared to adult size under ambient environmental conditions.

Constant Conditions

Data on weight gains showed that (1) females had higher weight gains than males, and (2) unshocked diploids had higher weight gains than the shocked groups (significance of "sex" and "ploidy", see Table 1 and Figure 3).

Data on length gains showed that (1) females had higher length gains than males, and (2) unshocked diploids had higher weight gains than the shocked groups (significance of "sex" and "ploidy", see Table 1 and Figure 3).

Data on condition factors showed that (1) females had higher condition factors than males, and (2) unshocked diploids had higher conditions factors than the shocked groups (significance of "sex" and "ploidy", see Table 1 and Figure 3).

Males had higher fillet yields than females (significance of "sex", see Table 1 and Figure 3).

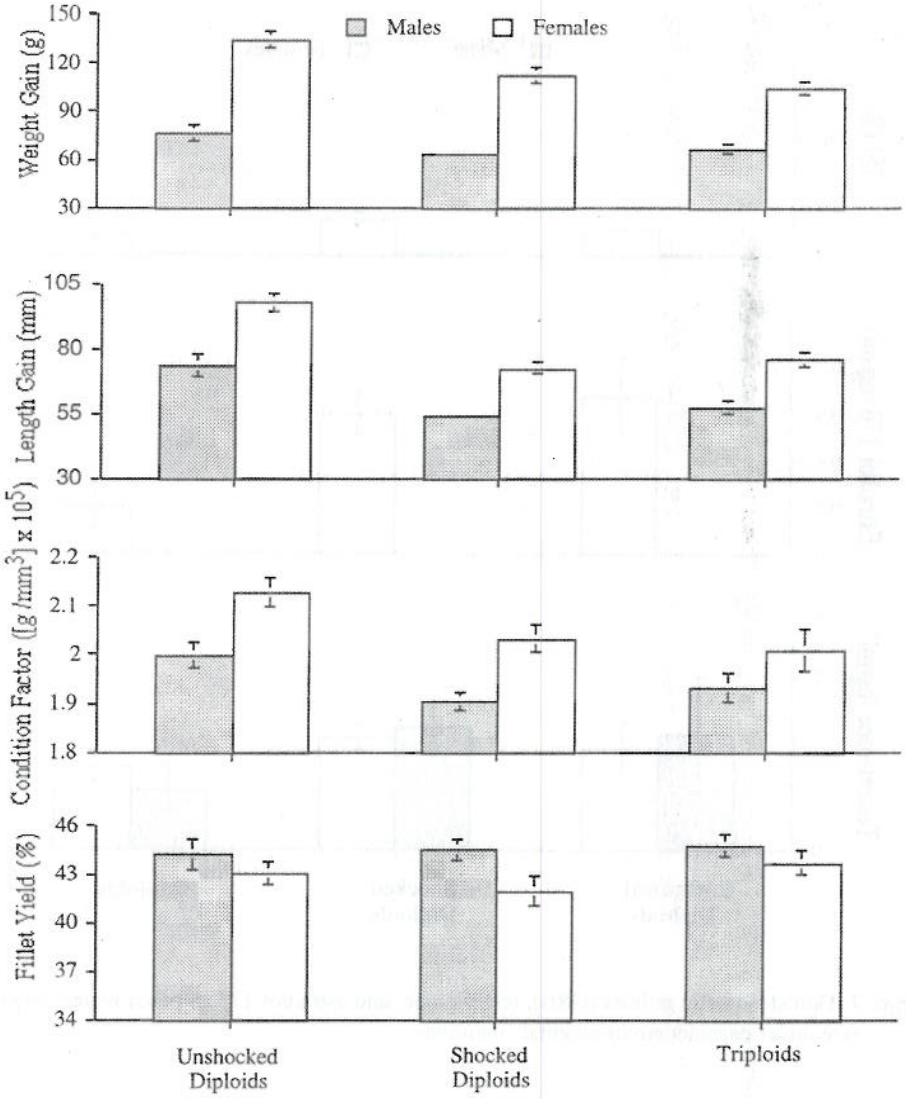

Figure 3. Weight gains, length gains, and condition factors in perch reared to adult size under constant environmental conditions.

Data on GSIs showed that (1) females had higher GSIs than males, (2) shocked diploids had higher GSIs than triploids, (significance of "sex", "ploidy" and "sex x ploidy"; see Table 1 and Figure 4).

There were no significant effects of either "sex" or "ploidy" on testosterone levels. Females had higher E_2 levels than males, and shocked female diploids had higher E_2 levels than triploid females, (significance of "sex", "ploidy" and "ploidy x sex; see Table 1 and Figure 4).

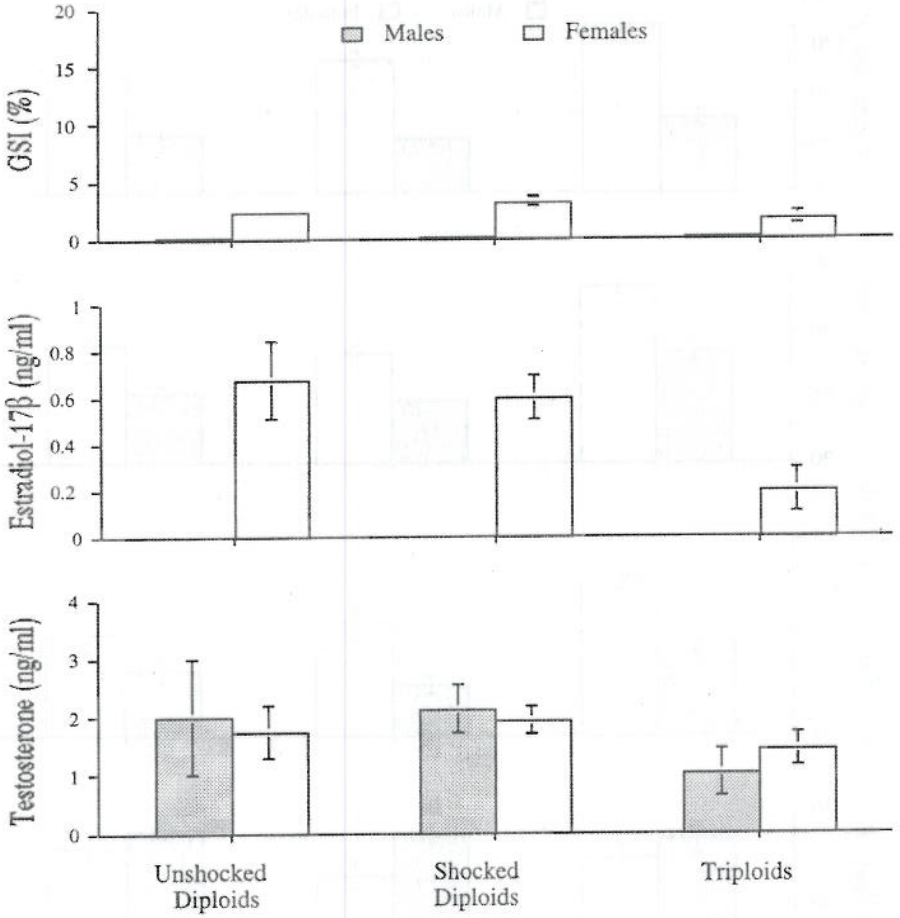

Figure 4. Gonadosomatic indices (GSIs), testosterone, and estradiol-17β in perch reared to adult size under constant environmental conditions.

Discussion

Our findings show that, for the aquaculture of yellow perch under ambient conditions (i.e., in ponds), triploids may have superior production traits than diploids if the negative effects of the shocks used to induce triploidy can be avoided. Because of the markedly faster growth of females versus males, the use of all-female stocks is a significant advantage for perch culture regardless of whether perch are reared under ambient or constant conditions. Under ambient conditions, however, females can exhibit a significant decline in fillet yield in conjunction with gonadal development. Our findings clearly show that triploids exhibit this problem to a far lesser degree than diploids, and may also have the potential to grow somewhat faster than diploids. For perch reared under constant environmental conditions, triploidy does not appear to offer any significant benefit.

Our findings also show that heat and hydrostatic pressure shocks applied to newly fertilized eggs exert a negative effect on the growth of perch that is independent of ploidy status, and that this effect continues beyond the juvenile stage until perch reach a market size of 100-150 g. We previously hypothesized that the negative effects of shocks on juvenile growth resulted from the biochemical actions of heat shock proteins (Malison et al., 1993b). HSPs are thought to prevent errors in transcription and translation, and through these and other mechanisms may protect organisms from the effects of severe stressors (Nagao et al., 1990). Heat and hydrostatic pressure shocks may also have non-specific actions on microtubules, which in turn could result in various mitotic aberrations including multipolar metaphases and aberrant cleavages (Garcia-Abiado, 1995). Supporting this idea are the findings that chum and masu salmon (*Oncorhynchus keta* and *O. masou*, respectively) embryos showed abnormal embryogenesis associated with chromosomes isolated from the nucleus, chromosome bridges, fragments, gaps, and rings among dividing cells (Yamazaki and Goodier, 1993). Regardless of the mechanism, our findings show that the effects of post-fertilization shocks are essentially permanent.

Triploid female perch (reared under ambient or constant conditions) had reduced serum E2 levels compared to diploids, a finding that has also been described in several other fish species (e.g., Lincoln and Scott, 1984; Benfey et al., 1989). In normal diploid perch, the stimulatory effects of ovarian estrogens on growth are responsible for much of the difference in growth between the sexes (Malison et al., 1985; Malison et al., 1988b). It is likely that ovarian estrogens also promote growth in triploid perch, and if so the reduced serum E_2 levels in triploids may (partially) offset any increased growth potential associated with their sterility.

One possible way of producing triploids without using shocks would be to cross fertile tetraploids with diploids. Experiments along this line are currently being conducted in our laboratory.

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References

Benfey, TJ, Dye, HM, Solar, II, Donaldson,, EM 1989 The growth and reproductive endocrinology of adult triploid Pacific salmonids. Fish Physiol. Biochem., 6: 113-120.

Calbert, HE 1975 Purpose of the conference. In: Aquaculture:Raising Perch for the Midwest Market. University of Wisconsin Sea Grant College Advisory Report No. 13, Madison, WI, pp. 7-8.

Carlander, KD 1977 Handbook of Freshwater Fishery Biology, Vol. 2. Iowa State University Press, Ames, IA, 431 p.

Garcia-Abiado, MAR 1995 Reproductive Biology and Chromosome Manipulation in the Nile Tilapia, *Oreochromis niloticus* Linnaeus. Ph.D. Thesis, University of Wales, Swansea, Wales, U.K., 382 pp.

Heidinger, RC and Kayes, TB 1986 Yellow perch. In: RR Stickney (Editor), Culture of Nonsalmonid Freshwater Fishes. CRC Press, Boca Raton, FL, pp 103-113.

Huh, HT 1975 Bioenergetics of Food Conversion and Growth of Yellow Perch (*Perca flavescens*) and Walleye (*Stizostedion vitreum vitreum*) Using Formulated Diets. Ph.D. Thesis, University of Wisconsin-Madison, Madison, 254 pp.

Le Cren ED 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in perch (*Perca fluviatilis*). J. Anim. Ecol., 20: 201-219.

Lincoln, RF and Scott, AP 1984. Sexual maturation in triploid rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol., 25: 385-392.

Malison JA, Kayes, TB, Held, JA, Barry, TP, and Amundson, CH 1993a Manipulation of ploidy in yellow perch (P*erca flavescens*) by heat shock, hydrostatic pressure shock, and spermatozoa inactivation. Aquaculture, 110: 229-242.

Malison, J.A., 1985. Growth Promotion and the Influence of Sex-Steroids on Sexually Related Dimorphic Growth and Differentiation in Yellow Perch (*Perca flavescens*). Ph.D. Thesis, University of Wisconsin-Madison, 153 pp.

Malison, JA and Held, JA 1992 Effect of fish size at harvest, initial stocking density and tank lighting conditions on the habituation of pond-reared yellow perch (*Perca flavescens*) to intensive culture conditions. Aquaculture, 104: 67-78.

Malison, JA, Best, CD, Kayes, TB, Amundson, CH and Wentworth, BC 1985 Hormonal growth promotion and eveidence for a size-related difference in response to estradiol-17β in yellow perch (*Perca flavescens*). Can. J. Fish Aquat Sci., 42: 1627-1633.

Malison, JA, Kayes, TB, Best, CD, Amundson, CH, Wentwoth, BC 1986 Sexual differentiation and use of hormones to control sex in yellow perch (*Perca flavescens*). Can. J. Fish Aquat Sci., 43: 26-35.

Malison, JA, Kayes, TB, Wentworth, BC, Amundson, CH, 1988a. Growth and feeding responses of male versus female yellow perch (*Perca flavescens*) treated with estradiol-17β. Can. J. Fish Aquat. Sci., 45: 1942-1948.

Malison, JA, Kayes, TB, Wentworth, BC, and Amundson, CH, 1988b Control of sexually related dimorphic growth by gonadal steroids in yellow perch (*Perca flavescens*). In: DR Idler, LW Crim, JM Walsh (Editors), Proceedings of the Third International Symposium on Reproductive Physiology of Fish. Memorial University of Newfoundland. St. John's, Newfoundland, Canada, p. 206.

Malison, JA, Procarione, LS, Held, JA, Kayes, TB, Amundson, CH, 1993b The influence of triploidy and heat and hydrostatic pressure shocks on the growth and reproductive development of juvenile yellow perch (*Perca flavescens*). Aquaculture, 116: 121-133.

Nagao, RT, Kimpel, JA, and Key JL 1990. Molecular and cellular biology of the heat-shock response. In: JG Scandalios and TRF Wright (Editors), Advances in Genetics, Vol. 28. Academic Press, New York, pp. 235-274.

Purdom, CE 1976. Genetic techniques in flatfish culture. J. Fish. Res. Bd. Can., 33: 1088-1093.

Schott, EF 1980. Sexually Dimorphic Growth in Young-of the-Year Yellow Perch (*Perca flavescens*) Under Controlled Environmental Conditions. M.Sc. Thesis. University of Wisconsin-Madison, Madison, 222 pp.

Sokal, RR, and FJ Rohlf, 1995. Biometry. The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Company, New York, 887 p.

Utter, FM, Johnson, OW, Thorgaard, GH and Rabinovitch PS 1983 Measurement and potential applications of induced triploidy in Pacific salmon. Aquaculture, 35: 125-135.

Yamazaki, F and Goodier, J 1993 Cytogenetic effects of hydrostatic pressure treatment to suppress first cleavage of salmon embryos. Aquaculture, 110: 51-59.