Aquaculture 285 (2008) 216–223

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of estradiol-17β on survival, growth performance, sex reversal and gonadal structure of bluegill sunfish *Lepomis macrochirus*

Han-Ping Wang [a,*], Zexia Gao [a,c], Beatrix Beres [a,1], Joseph Ottobre [b], Geoff Wallat [a], Laura Tiu [a], Dean Rapp [a], Paul O'Bryant [a], Hong Yao [a]

[a] Aquaculture Genetics and Breeding Laboratory, Ohio State University South Centers, 1864 Shyville Road, Piketon, Ohio 45661, USA
[b] Department of Animal Sciences, Ohio State University, 2027 Coffey Road, Columbus, OH 43210 USA
[c] College of Fishery, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan, Hubei, 430070, PR China

ARTICLE INFO

Article history:
Received 21 February 2008
Received in revised form 16 August 2008
Accepted 26 August 2008

Keywords:
Estradiol-17β
Sex reversal
Growth performance
Gonadal structure
Lepomis macrochirus

ABSTRACT

We systematically investigated the feminization of bluegill *Lepomis macrochirus* by oral administration of various doses of estradiol-17β (E_2) and evaluated their effects on the growth performance, production and gonadal structure of sex-reversed female bluegill at both sex ratio and histological levels. With positive control treatment, 30-day-old fry were fed with E_2 at 50, 100, 150 and 200 mg kg^{-1} diet for 60 days. The survival of fish in the E_2 treated and control groups was not significantly different ($P>0.05$). The growth of the treated fish was significantly retarded during the period of treatment, while there was no side effect detected post-treatment and the retarded fish caught up during 120 days of culture after E_2 treatment. All the treated groups produced 100% monosex female populations based on the macroscopic shape of gonads, and there were no significant differences detected between any E_2 treatment and control group in the mean GSI of females during the spawning season from June to October ($P>0.05$). Histologically, 13.3% and 5.0% of the intersex fish were determined to come from the 50 and 100 mg kg^{-1} E_2 treatment groups, respectively, with 6.9% and 4.1% of the gonadal area containing spermatocytes. Most of genotypical male fish treated with exogenous E_2 developed gonadal structures histologically indistinguishable from the gonads of females. This study suggests that 150 mg kg^{-1} E_2 is the optimal dosage for feminization in bluegill, with 50 and 100 mg kg^{-1} E_2 being sub-optimal and 200 mg kg^{-1} E_2 being over-optimal.

1. Introduction

The bluegill sunfish *Lepomis macrochirus* is currently recognized as one of the most valuable North American recreational fish. Bluegill and its F_1 hybrids (female green sunfish *L. cyanellus* × male bluegill) have long been commercially cultured to support recreational fishery stocking needs throughout the middle south, and southeastern United States (Brunson and Robinette, 1986; Tidwell and Webster, 1993; Brunson and Morris, 2000). The recreational market offers a great opportunity for aquaculture of bluegill and its hybrid. The current cost of catch-and-keep and catch-and-release bluegill fishing runs about $30 for 10 fish or $29 for 1 h of fishing (Fields et al., 2004). However, a common problem in small impoundments and farm ponds stocked with this species is overcrowded bluegill population due to their prolific reproductive nature and early maturation (Mitzner, 1984; Arslan, 2002). Control of the bluegill population density through traditional management techniques based on establishing a prey–predator balance is not always successful (Clark and Lockwood, 1990; Arslan, 2002) because of the dynamic nature of the factors that affect reproduction, recruitment and growth (Kohler and Kelly, 1991). An alternative approach to the management of bluegill in small impoundments is to stock monosex populations. Monosex populations can eliminate the problem of prolific reproduction, precocious maturity and their consequences (Dunham, 1990).

Bluegill sunfish have become an increasing-economically important and high-value species in aquaculture. In some states, like Ohio and Michigan in the United States, bluegill have been listed as one of the top three culture species of fish because of their desirable characteristics (i.e., rapid growth and efficient feed conversion during summer, ready acceptance of commercial diets and low requirements to water quality) for production (Lewis and Heidinger, 1978; McLarney, 1987; Ehlinger, 1989), the demand for them and their high value in the marketplace. Because the aquaculture businesses in some regions in the U.S. do not appear to be able to economically sustain large market products (e.g., catfish), regional niche marketing of high-value products will be an important business model in the future. This is due to the fact that demand for these high-value species has remained high in regional niche markets and there are no imported fish products comparable to these unique, high-value species.

* Corresponding author. Tel.: +1 740 289 2071; fax: +1 740 289 7491.
E-mail address: wang900@ag.osu.edu (H.-P. Wang).
[1] Current address: Department of Fish Culture, Szent Istvan University Faculty of Agriculture and Environmental Sciences, 1 Pater K. Street, Godollo H-2103, Hungary.

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.08.041

For example, retail prices for bluegill reached to $29.00–35.00/kg as compared to $10/kg retail for catfish (Jungle Jim's market, Cincinnati, Ohio, personal communication) and $8–12/kg for fresh tilapia fillets (Lutz et al., 2003). Despite this opportunity, rapid expansion of the bluegill aquaculture industry has not occurred in this country. One reason in particular hindering expansion has been the relatively slow growth of currently cultured populations of this species.

Recently, a number of studies have shown that bluegill possess the inherent capacity to grow to food market sizes substantially faster than hybrid bluegill (Hayward and Wang, 2002). Male bluegill, in particular, appear to hold the greatest potential for the food market due to their more rapid growth capacity relative to females (Hayward and Wang, 2006). Male bluegill outweighed female bluegill by 111.9% at the end of a 300-d experiment, and were also significantly larger than both male and female hybrid bluegill that were reared in parallel (Hayward and Wang, 2006). In this study, fish were held individually and growth was assessed by comparing the means of eight replicates. The follow-up research results we achieved recently on evaluation of growth performance of mostly-male group versus mixed-sex group indicated that male bluegill communally reared in groups were still able to grow faster than mixed or mostly-female populations in commercial aquaculture settings (Wang et al., in press). This indicated that social interaction costs among grouped male bluegills, which are capable of impeding their growth (Hayward and Wang, 2006), were not so great as to override the higher growth capacity of male bluegills. These findings suggest that monosex male culture will hold considerable potential as a method to increase the efficiency and profitability of bluegill aquaculture by improving growth rates.

Monosex culture has been applied in several species in order to improve fish production (Schreck, 1974; Donaldson and Hunter, 1982; Hunter and Donaldson, 1983; Yamazaki, 1983). The potential advantages sought from these approaches include one or more of the following features: achievement of a higher growth rate, elimination of reproduction, reduced variation in harvest size, and reduction of risk of environmental impact resulting from the escape of exotic species. The investment in gonads can commonly account for 20–30% of a fish's energy expenditure, and culture of males and females together results in early and frequent reproduction (Mires, 1995). This is especially true for bluegill (Emig, 1966; Bennett, 1971).

Monosex populations can be achieved in two different ways, directly through hormonal sex reversal or indirectly through breeding of sex-reversed fish (Hunter and Donaldson, 1983; Dunham, 1990; Tave, 1993; Kim et al., 1997; Piferrer, 2001; Park et al., 2004). However, the indirect technique has the best potential for future applications because it allows for production of monosex populations in mass numbers by a simple breeding process (Piferrer, 2001), eliminates the need for continued use of hormones and the fish that reach the marketplace have never been exposed to steroids (Arslan and Phelps, 2004). Therefore, both in male and female heterogametic species, the first step to the production of monosex male populations through breeding of sex-reversed fish is to achieve feminization of genotypic males (Piferrer, 2001). When females with a male genotype (i.e., XY or ZZ) are bred to males, a higher percentage of males will result (XY♀×XY♂ → 1/4 XX♀, 1/2 XY♂, 1/4 YY♂ or ZZ♀×ZZ♂→100% ZZ♂). In heterogametic male species, YY♂ offspring used as broodstock would result in 100% ♂ offspring.

Among the sex-reversal reports in bluegill, initial attempts to masculinize bluegill by oral administration of methyltestosterone were unsuccessful (Chew and Stanley, 1973). Al-Ablani and Phelps (2002) also found a predominance of intersex fish and reduction in survival of both males and females in the oral administration treatments of trenbolone acetate and 17α-methyltestosterone. Methods to feminize bluegill, however, have been relatively more successful. Ninety-nine percent of female bluegill populations as determined from the macroscopic shape of the gonads were obtained by feeding fry a diet with 100 mg kg^{-1} and 200 mg kg^{-1} estradiol-17β (E_2) diet for 30–45 days in two separate trials (Al-Ablani, 1997; Arslan and Phelps, 2004). Al-Ablani (1997) also reported that the administration of estrogens via short time periodic immersions was found to be less effective (Al-Ablani, 1997; Arslan and Phelps, 2004).

Estradiol-17β, a natural estrogen, has been shown to be an effective feminization hormone in Cyprinidae, Anabantidae, Poeciliidae, Ictaluridae, Salmonidae and Cichlidae (Pandian and Sheela, 1995). Information on survival, growth and reproduction of hormonally sex-reversed fish is as important as information on achievement of hormonal induction of sex reversal. E_2 has been reported to affect survival negatively in many fish species if a particular threshold is surpassed, such as in coho salmon *Oncorhynchus tshawytscha* (Hunter et al., 1986), zebra cichlid *Cichlasoma nigrofasciatum* (George and Pandian, 1996), mud loach *Misgurnus mizolepis* (Kim et al., 1997). The negative effects on survival depend on a number of factors, the treated species, treatment timing and treatment intensity being the most important ones. One of the objectives of inducing hormonal sex reversal is to realize 100% growth potential. Some synthetic steroids have been proven to promote growth (McBride and Fagerlund, 1973; Fagerlund and McBride, 1975). In theory, growth enhancement can occur through improvement in appetite, digestion, absorption of metabolic re-arrangements leading to overall anabolism, and the contribution of each of these factors varies somewhat among different species and type of steroid used (Woo et al., 1993). For example, E_2 promoted growth in yellow perch *Perca flavescens* by stimulating appetite (Malison et al., 1988). On the other hand, E_2 has been proven to depress growth in many fish species (Funk et al., 1973; Goetz et al., 1979; Johnstone et al., 1978; Blázquez et al., 1998). Hormonally sex-reversed females may not be functionally equal to genetic females. The sequence of increasing effects of estrogens on the gonadal histology of fish, from an unaffected testis to a fully feminized fish has been documented (Piferrer and Donaldson, 1992). The appearance of intersex fish is often observed after treatment with sex steroids. This may be the result of incomplete treatment because of the improper combination of treatment variables or because the species treated responds poorly to exogenous steroids (Piferrer, 2001).

Although there have been some reports about sex reversal in bluegill, no systematic study referred to the effect of different doses of estradiol-17β on bluegill survival, growth during and after E_2 treatment, sex ratio and gonadal structure. The objectives of our present study were to evaluate the effectiveness of various estradiol-17β treatments on the growth performance, production and gonadal structure of sex-reversed female bluegill at both sex ratio and histological levels through a bluegill breeding program.

2. Materials and methods

2.1. Fry production

The female broodfish were selected from a group of fish that was derived from a wild population in northern Ohio in 2004. The males were selected from a population caught from a lake in southern Ohio in 2003. Eight males and four females were selected from the two populations housed in the Aquaculture Wet Laboratory, Ohio State University South Centers, tagged with PIT tags and stocked into two tanks with flow-through well water at a ratio of two females to four males per tank in 2005. Fish were fed daily at 2% of body weight with a high-protein feed (Silver cup, 45% crude protein, 16% crude fat). Two artificial spawning nests were placed in each tank at mating, and were checked by hooking them out of the water and cleaned every morning. Nests with eggs were placed in the bottom of aerated 100-L tanks with flow-through well water for incubation. Eggs hatched in 24–36 h at 24–26 °C; and newly hatched larvae were reared in 70-L round tanks with flow-through well water at 23 °C for 4 weeks before the feminization experiment. Prior to hormone treatments, fry were gradually weaned from brine shrimp to a dry diet.

Table 1
Effects of various dosages of estradiol-17β (E_2) on survival, food consumption (FC), E_2 consumption (E_2C), body length (BL), and body weight (BW) of *L. macrochirus* during treatment period

E_2 dose (mg kg^{-1})	Survival (%)	FC (g fish^{-1})	E_2C (μg fish^{-1})	Mean final BL (cm)	Mean final BW (g)
0 (control)	62.5±8.43^a	0.378±0.023^a	0	1.71±0.15^a	0.103±0.31^a
100	61.0±8.76^a	0.429±0.010^b	42.98±0.97	1.61±0.14^b	0.087±0.22^b
150	58.25±7.59^a	0.547±0.045^c	82.07±6.82	1.63±0.20^b	0.089±0.33^b
200	54.75±4.65^a	0.447±0.014^b	89.50±2.85	1.52±0.15^c	0.070±0.20^c

Means (±SE) within a column followed by different superscript letters were significantly different ($P<0.05$).

2.2. Feminization experiment

The day before the experiment, the E_2 diets were prepared. The desired quantities of estradiol-17β (E_2) were dissolved in 400 ml ethanol kg^{-1} feed. The addition of E_2 to the feed was carried out by slowly pouring the hormone solution onto feed while mixing with an electric mixer and then the feed was put into a fume hood to allow the alcohol to evaporate. The control diet was prepared in the same manner using alcohol without hormone. When fry were 4 weeks of age, with 13.9±1.3 mm mean body length and 0.39±0.09 mg mean body weight, 100 fish were randomly assigned to each of twenty 25-L round tanks. The control plus four treatment groups made a total of 5 groups, each having four replicates. All fry came from the same batch for the control group and the 100, 150, 200 mg kg^{-1} E_2 treatments. Fry from a different batch were used for 50 mg treatment group because there were not enough fry for all five treatments at the beginning of the experiment. Fry in all treatment groups received their ration of E_2 feed 5 times daily from day 30 for 60 days. All fish were fed to satiation during the experiment.

Mortality was monitored daily in each experimental group excluding the 50 mg kg^{-1} E_2 group during the experiment, from the 30th to 90th day post-hatch (dph). Twenty fish were sampled from each tank for body weight and body length at 90 dph. Feeding amounts were monitored and recorded weekly excluding 50 mg kg^{-1} group, by subtracting remaining feed amount after the last feeding from the previous feed amount in the container, for calculation of food consumption (FC), E_2 consumption (E_2C), and gross growth efficiency (GGE). During the experiment, daily mean (±SD) water temperature and dissolved oxygen concentrations were 21.5±0.5 °C and 7.8±0.4 mg L^{-1}, respectively, and water flow rate was 0.32 L/min.

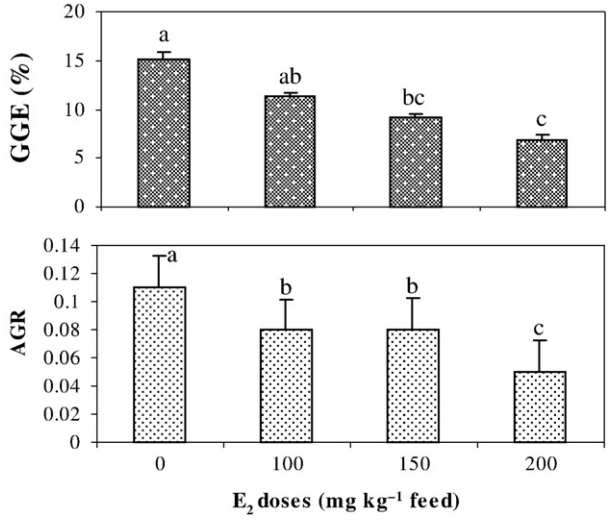

Fig. 1. The absolute growth rate (AGR) and gross growth efficiency (GGE) of *L. macrochirus* during E_2 treatment. The groups with the same letter were not significantly different ($P>0.05$).

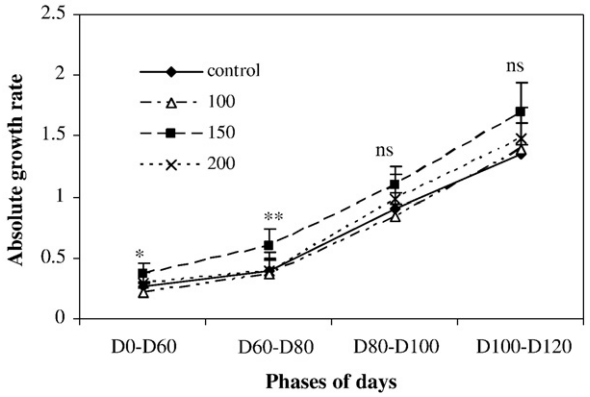

Fig. 2. The absolute growth rates of *L. macrochirus* post-treatment. NS, no significant difference ($P>0.05$) between different E_2 treated and control groups; *, significant difference ($P<0.05$) between 150 and (control+100) groups; **, significant difference ($P<0.05$) between 150 and (control+100+200) groups.

2.3. Post-feminization experiment

The growth of the fish in 100, 150, 200 mg kg^{-1} E_2 treatment groups and the control group was monitored for 120 days after the E_2 treatment. During the first 60 days post-E_2-treatment, all fish were maintained in the original 25-L round tanks utilized for the feminization experiment (each group had four replicates). The mean water temperature and dissolved oxygen concentrations were 19.5±1.3 °C and 7.6±0.6 mg L^{-1}, respectively, and water flow rate was 0.32 L/min. At day 61 post-E_2-treatment, when fish biomasses approached carrying capacities in some tanks, fifty fish, randomly sampled from each treatment (100 mg, 150 mg, and 200 mg kg^{-1} E_2) and the control group, were weighed and stocked into each of sixteen 55-L round tanks. Fish were fed to satiation 3 times daily with fry feed (Silver Cup, 52% crude protein, 14% crude fat). Feed amounts were monitored and recorded daily for calculation of FC and GGE by subtracting remaining feed amount after the last feeding from the previous feed amount in the container. The mean water temperature and dissolved oxygen concentrations were 23.4±2.5 °C and 7.3±0.6 mg L^{-1}, respectively, and water flow rate was kept at a level of 0.41 L/min for all tanks. Tanks were cleaned regularly to maintain good water quality. Twenty fish were sampled from each tank for body weight every 20 days.

Samples of 15–20 fish in each E_2 treated group (47 fish for 100 mg group) were anaesthetized with MS-222 at the age of 20–24 months and the sex ratio of each treatment was determined by macroscopic and microscopic examination of gonadal tissue by the gonad squash method (Guerrero and Shelton, 1974). In addition, a single gonad from

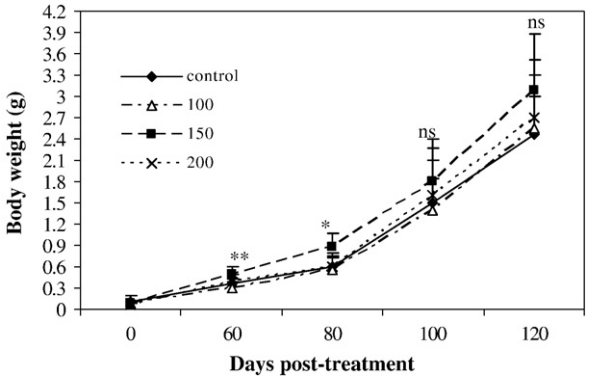

Fig. 3. The growth of *L. macrochirus* post-treatment. NS, no significant difference ($P>0.05$) among different E_2 treated and controlled groups; *, significant difference ($P<0.05$) between 150 and (control+100) groups; **, significant difference ($P<0.05$) between 150 and (control+100+200) groups.

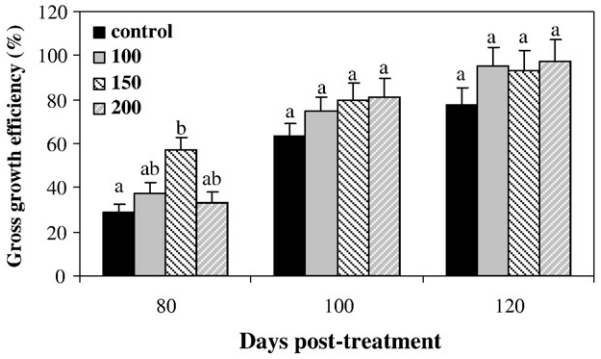

Fig. 4. The gross growth efficiency of *L. macrochirus* post-treatment. At any given date, the groups with the same letter were not significantly different ($P>0.05$).

15–20 of the fish from each of the E_2 treated groups was submitted for histological analysis to confirm the results of the squash method (Park et al., 1998). In the control group, samples of 115 fish were examined for sex ratio by macroscopic method. At the same time, the gonads of 20 females and 10 males were assessed histologically.

2.4. Histological preparation and analysis

For histological observation, adult fish were randomly sampled from each treatment and control group during the spawning season in 2007. The fish were deeply anaesthetized with MS-222 and the gonads were dissected. Body weight and gonad weight were recorded for calculation of the gonadosomatic index (GSI; gonad weight expressed as percentage of the body weight). The gonads from each fish were fixed with Prefer and processed for histological sectioning by routine dehydration and paraffin embedding procedures. For each fish, at least five cross-sections (4–6 μm) covering different portions of the gonad were cut using a Reichert-Jung 820-II microtome, stained with Mayer's hematoxylin and eosin phloxine B solution, examined and microphotographed to determine their phenotypic sex (Park et al., 1998). For intersex fish, the proportions of spermatocytes and oocytes were calculated by approximating the area with spermatocytes and oocytes on 10 slices of tissue.

2.5. Data analysis

Differences in the mean responses among treatments in survival, growth, FC, E_2C and GSI were determined with one-way ANOVA followed by Duncan's test using SAS program (SAS Institute, 1988). E_2C at each dose was calculated by the formula $E_2C=FC\times E_2$ dose. Absolute growth rate (AGR) was calculated according to the formula $AGR=(W_{t2}-W_{t1})/T\times 100$. Where W_{t1} and W_{t2} are fish weight at the start and end of a growth period respectively, and T is the time in days between weighing.

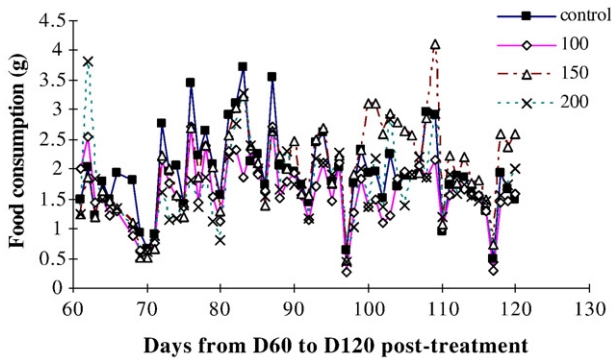

Fig. 5. The feed consumption of *L. macrochirus* from D60 to D120 after E_2 treatment.

Table 2
Effects of various doses of estradiol-17β (E_2) on sex ratios in *L. macrochirus*

E_2 dose (mg kg^{-1})	Macroscopic (shape of gonad)		Microscopic (histological structure)			
	N	Female(%)	*N*	Female (%)	No. of intersex fish	Area containing testicular tissue in the intersex fish (%)
0 (control)	115	66.1	20[a]	100	0	–
50	15	100	15	86.7	2	6.9
100	47	100	20	95.0	1	4.1
150	20	100	20	100	0	–
200	20	100	20	100	0	–

[a] The gonads were from the female examined by macroscopic method.

Gross growth efficiency (GGE) was calculated according to the formula $GGE=(W_{t2}-W_{t1})/FC\times 100$.

3. Results

3.1. Survival

There was no significant difference detected among treatments, and between any treatment and the control group in survival (Table 1) ($P>0.05$). No signs of toxicity or behavioral differences between treatment groups and control fish were observed during and after the treatment.

3.2. Growth and growth efficiency

The feed consumption, E_2 consumption, and body length and weight during treatment are presented in Table 1. The fish in the E_2 treated groups consumed more feed but grew significantly slower than the control fish. The final body length and weight of the groups treated with 100 and 150 mg kg^{-1} E_2 were significantly lower than those of control ($P<0.05$). A further growth depression occurred in the 200 mg kg^{-1} E_2 group ($P<0.05$). The absolute growth rate and gross growth efficiency of the control fish were significantly higher ($P<0.05$) than those of all E_2 treated groups (Fig. 1). Among the E_2 treated groups, the fish in the 200 mg kg^{-1} E_2 treatment had significantly lower AGR and GGE than the 100 and 150 mg kg^{-1} E_2 groups (Fig. 1).

There were no significant differences ($P>0.05$) detected among E_2 treated and control groups during the 120 days post-treatment in body weight and growth rates, except that the fish in 150 mg kg^{-1} E_2 treated group had significantly heavier body weight and higher growth rates at days 60 and 80 post-treatment (Figs. 2 and 3).

Similar to growth rate, no significant difference ($P>0.05$) was detected among E_2 treated and control groups during 120 days post-treatment experiment in gross growth efficiency and food consumption (Figs. 4 and 5), although a significantly higher GGE was detected

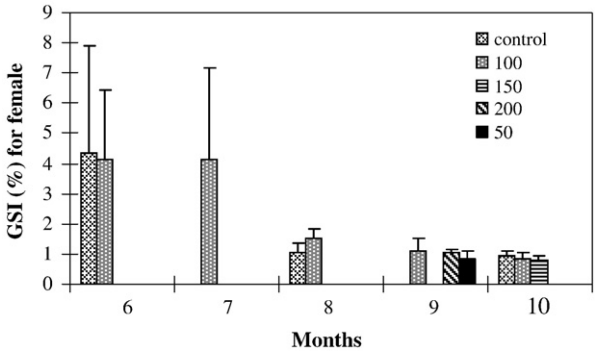

Fig. 6. The gonadosomatic index (GSI) of *L. macrochirus* females in control and E_2 treated groups from June to October (spawning season). NS, no significant difference ($P>0.05$) between control and E_2 treated groups.

at day 80 only for the fish in 150 mg kg^{-1} E_2 treated group compared to control fish (Fig. 4).

3.3. Sex ratio and GSI

All of the treatment groups of 50, 100, 150 and 200 mg kg^{-1} E_2 yielded 100% monosex female populations as determined from the macroscopic shape of the gonads. However, using histological method, 13.3% (2 out of 15) and 5.0% (1 out of 20) intersex fish were identified in the groups of 50 and 100 mg kg^{-1} E_2, respectively. Of the 115 fish sampled from the control group, 66.1% were identified as females (Table 2).

Gonadal development of reversed females in all the treatment groups appeared normal, and there were no significant differences detected between any E_2 treatment and control group in the mean GSI of females during the spawning season from June to October ($P>0.05$). The GSI of females in treatment and control groups was the highest in June to July, and then gradually decreased through October (Fig. 6).

3.4. Histological structure

Histological structure of the gonads from all the E_2 treatment groups showed the same pattern as that of gonads from the control group during spawning season (Fig. 7). Similar to that in the control fish (Fig. 7a), histological structure of the gonads from the treatment groups showed that females had ovaries with a lot of large fully grown oocytes containing a large amount of acidophilic yolk fluid filling the

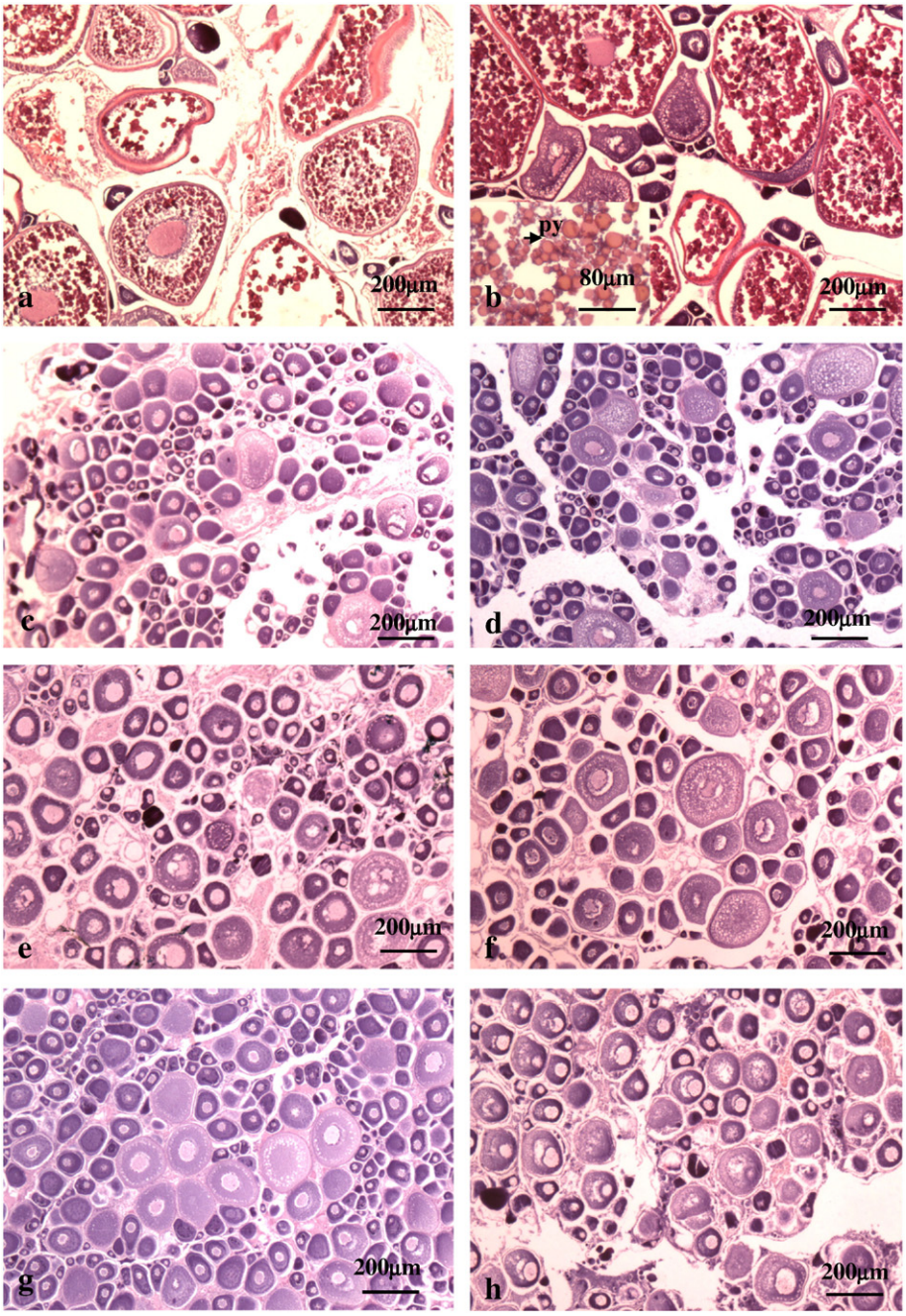

Fig. 7. Transverse sections of gonads of adult female *L. macrochirus*. (a) Ovary of control fish in June. (b) Ovary of 100 mg kg^{-1} E_2 treated fish in June, inset: acidophilic yolk granules (py) viewed under high power. (c) Ovary of control fish in August. (d) Ovary of 50 mg kg^{-1} E_2 treated fish in August. (e) Ovary of 100 mg kg^{-1} E_2 treated fish in August. (f) Ovary of 200 mg kg^{-1} E_2 treated fish in August. (g) Ovary of control fish in September. (h) Ovary of 150 mg kg^{-1} E_2 treated fish in September.

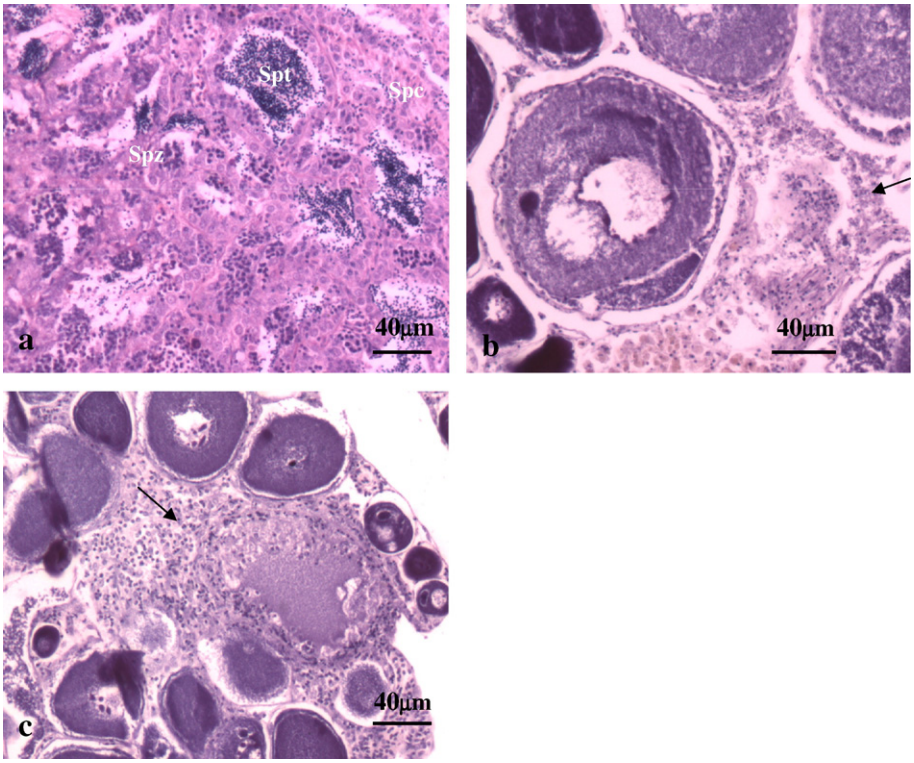

Fig. 8. Transverse sections of gonads of adult male and intersex *L. macrochirus*. (a) Testis containing spermatocytes (Spc), spermatids (Spt), spermatozoa (Spz) from control fish. (b) Intersex gonad from fish treated with 50 mg kg^{-1} E_2, showing spermatocytes (arrow) in the middle of the numerous oocytes. (c) Intersex gonad from fish treated with 100 mg kg^{-1} E_2, showing spermatocytes (arrow) in the middle of the numerous oocytes.

cytoplasm in June (Fig. 7b). In August and September, gonads from the treatment groups were filled with perinucleolar, previtellogenic, vitellogenic stage and fully grown oocytes as were the gonads from the control group (Fig. 7c–h). In the intersex fish from E_2 treatment groups of 50 mg and 100 mg, some spermatocytes similar to those in the control males (Fig. 8a) were observed among the numerous oocytes in a few gonads from the two treated groups (Fig. 8b and c). The testicular tissue accounted for approximately 6.9% of the area of the histological slices in the intersex fish from the 50 mg group and 4.1% from the 100 mg group. Therefore, most gonadal tissue of reversed females was indistinguishable from ovarian tissue of genotypic females in histological structure. There were no intersex gonads identified from females in the 150 and 200 mg treatment groups.

4. Discussion

The use of estrogens for the production of monosex female stocks in finfish aquaculture has increased in recent years. Estrogens have been utilized for controlling sex differentiation in at least 56 different species of teleosts belonging to 24 different families (Piferrer, 2001). Several members of the sunfish family, Centrarchidae, such as largemouth bass *Micropterus salmoides*, black crappie *Pomoxis nigromaculatus*, and bluegill *L. macrochirus* have been evaluated for application of monosex population techniques. In black crappie and largemouth bass, females grow faster and larger than males (Arslan, 2002). A 100% female population of largemouth bass was produced by feeding 40-day-old fry with a 100–400 mg kg^{-1} E_2 diet for 40 days (Al-Ablani and Phelps, 2001). Feeding 33-day-old black crappie fry a 200 mg kg^{-1} diethylstilbestrol diet for 30 days resulted in low survival (9–16%) and yielded 59–100% female populations in a 48% genetic female experimental population (Al-Ablani and Phelps, 1997). Sex revered females of black crappie were also produced by immersing fry into a 1 mg L^{-1} E_2 solution every 3 to 5 days for 5 h on ten occasions between 45 and 86 days of age. This treatment regimen produced 71% females from a 42% female experimental population (Arslan, 2002). In bluegill, since males grow faster than females (Arslan, 2002; Hayward and Wang, 2006), initial attempts to masculinize the species by oral administration of methyltestosterone (Chew and Stanley, 1973; Al-Ablani and Phelps, 2002) and trenbolone acetate (Al-Ablani and Phelps, 2002) were not successful. Even if they had been successful, the marketing of fish that were treated during early development with sex steroids would be difficult due to lack of consumer acceptance. Feminization is the first important step for producing all-male populations through selective breeding (Yamamoto, 1964, 1975; Johnstone et al., 1979; Chevassus et al., 1988; Scott et al., 1989; Varadaraj and Pandian, 1989; Kavumpurath and Pandian, 1992a,b; Onozato, 1993; Liu et al., 1996). While feminization of bluegill populations has been achieved by feeding 30-day-old fry a 100 mg kg^{-1} E_2 diet for 30 days (Al-Ablani, 1997) and a 99% feminization rate in a 39% female experimental population was produced by feeding 13.8 mm fry a 200 mg kg^{-1} E_2 diet for 45 days (Arslan and Phelps, 2004), there has been no systematic study of the effect of estradiol-17β on survival and growth during and after E_2 treatment, with a concomitant examination of sex ratio and gonadal structure in sunfish.

In the present attempt to feminize bluegill through the dietary administration of a natural steroid E_2, we found that doses of 150 and 200 mg kg^{-1} E_2 diet induced the production of 100% females in a 66.1% genetically female experimental population; no evidence of intersex fish was found. Lower treatment doses of 50 and 100 mg kg^{-1} E_2 diet produced populations that appeared to be 100% females from the macroscopic shape of the gonads; however, upon histological examination were found to contain 13.3% and 5.0% intersex fish with sections of their gonads containing spermatocyte accounting for 6.9% and 4.1% of the total gonad area, respectively. The intersex fish found in the 50 and 100 mg treatment groups may result from the hormone dose or treatment period being inadequate. Arslan and Phelps (2004) fed 13.8±0.6 mm fry a 200 mg kg^{-1} E_2 diet for 45 days, which resulted in 0.7% of the fish being intersex. In the present study, no intersex fish were found in the 150 mg and 200 mg kg^{-1} E_2

treatment groups when fed for 60 days. This may be the result of a prolonged treatment period in our experiment.

Both male and female skewed sex ratios have consistently been observed in natural and experimental bluegill populations (Schmittou, 1967; Al-Ablani, 1997; Arslan, 2002). Arslan and Phelps (2004) found in their feminizing experiment that 61.2% of the control group was male. In reverse, 66.1% of our control group was female. There have been indications that both genetic and environmental factors are involved in sex determination of bluegill (Al-Ablani, 1997). Therefore, the difference of the initial sex ratios in these two experiments should not be surprising.

In bluegill, feminizing genotypic males appears to occur with a much higher success rate when compared to masculinizing females (Chew and Stanley, 1973; Al-Ablani and Phelps, 2002). Explanations for this apparent anomaly may include that the process of ovarian differentiation, corresponding to the period at which the developing gonads would be responsive to steroids, may occur over a longer interval of time relative to that of testicular differentiation, or that bluegill gonadal tissue may be more responsive to estrogen treatment compared to androgen treatment.

In our study, the genotypic male fish treated with exogenous E_2 developed gonadal structures indistinguishable from the gonads of genotypic females except for the intersex fish. The phenotypic females developed mature eggs as shown in histological sections.

Fish in E_2 treated groups grew significantly slower than control fish during the period of treatment, indicating significant growth depression of E_2, especially in the high dose of 200 mg group. This result is consistent with most of previous reports. Goetz et al. (1979) found a dose-dependent decrease in the size of coho salmon treated with high E_2 concentrations. Funk et al. (1973) in pink salmon *O. gorbuscha*, Johnstone et al. (1978) in rainbow trout *O. mykiss*, and Blázquez et al. (1998) in European sea bass *Dicentrarchus labrax*, have also reported E_2-related growth depression. However, estrogen promoted growth in yellow perch and Japanese eel *Anguilla japonica* (Malison et al., 1988; Satoh and Nimura, 1991). Woo et al. (1993) also reported that E_2 administered in the diet did not affect the growth and appetite in the red sea bream *Chrysophrys major*. The higher feed consumption of the fish in E_2 treated groups during the period of treatment in our study might indicate that E_2 could stimulate the appetite of fish. The administration of E_2 to immature fish is known to induce some of the physiological changes occurring in sexually maturing teleosts, including those at the metabolic level in the liver (Mommsen and Walsh, 1988). The influence of oral administration of E_2 on digestion and metabolism in bluegill, as well as the mechanism for these, need to be further studied.

As many authors have not chosen to undertake long-term studies on the growth of sex-reversed fish, available information on the post-treatment growth of estrogen treated fish is scanty and inconsistent (Pandian and Sheela, 1995). The experiments undertaken by George and Pandian (1998) on black molly *Poecilia sphenops* treated with different doses of 17α-methyltestosterone estimated the weight gain as a measure of growth in treated (for a period of 1 month from birth) and control individuals at the age of 3, 6, 9, 15, and 18 months. The steroid enhanced relative growth in 3-month-old individuals when administered in doses up to the pre-optimal level, beyond which the increase in relative growth began to diminish. All studies about sex-reversal in bluegill did not refer to its growth after the estrogen or androgen treatments. Our findings show that in spite of the growth loss during the period of treatment, body weight of E_2 treated fish eventually caught up; the growth rates of fish in most of the E_2 treated groups were higher to some degree than those in the control group during the 120 days post-E_2-treatment experiment. By 120 day post-treatment, there was no significant difference ($P>0.05$) between E_2 treated and control groups in body weight and growth rate. We speculated compensatory growth (CG) occurred in the E_2 treated fish during the period of post-treatment. Compensatory growth is described as a phase of accelerated growth, commonly seen when favorable conditions are restored after a period of growth depression (Ali et al., 2003). The factors that can induce CG include periods of feed restriction and re-feeding, low temperatures, hypoxic conditions, salinity, prophylactic treatment, density alternation and sterilization treatment (Ali et al., 2003). In our study, the growth of the E_2 treated fish was restrained by estrogen treatment, but compensation (or perhaps over-compensation in the case of the 150 mg kg^{-1} E_2 treatment group) seemed to occur after cessation of the E_2 treatment. This is perhaps the first time that compensatory growth of sex-reversed fish has been reported, but this is in need of further study and confirmation.

In summary, this study demonstrates that oral administration of E_2 to bluegill successfully induces sex differentiation in favor of females. Moreover, considering all the effects of E_2 on survival, growth (during and after E_2 treatment), and sex ratio, we conclude that the E_2 dose of 150 mg E_2 kg^{-1} feed is the optimal dosage for feminization in bluegill, with 50 and 100 mg kg^{-1} E_2 being sub-optimal and 200 mg kg^{-1} E_2 over-optimal. Further study is also required to produce all-male progeny by employing sex-reversed broodstock and to understand the sex determination mechanism by using offspring testing in the bluegill.

Acknowledgements

This study was supported by grants provided to the Ohio State University Research Foundation from the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, under Agreement Nos. 2003-38879-02091 and 2004-38879-02192. Salaries and research support were provided by state and federal funds appropriated to The Ohio State University, Ohio Agricultural Research and Development Center. We thank Russ MacDonald and Christina Leighfield for their assistance in managing the experiments.

References

Al-Ablani, S.A., 1997. Use of synthetic steroids to produce monosex populations of selected species of sunfishes (Family: Centrarchidae). Ph.D. dissertation, Auburn University, Auburn, AL, USA.

Al-Ablani, S.A., Phelps, R.P., 1997. Sex reversal in black crappie *Pomoxis nigromaculatus*: effect of oral administration of 17a-methyltestosterone on two age classes. Aquaculture 158, 155–165.

Al-Ablani, S.A., Phelps, R.P., 2001. Induction of feminization in largemouth bass (*Micropterus salmoides*) by oral administration of estradiol-17 beta or diethylstilbestrol and associated pathological effects. Journal of Aquaculture in the Tropics 16, 185–195.

Al-Ablani, S.A., Phelps, R.P., 2002. Paradoxes in exogenous androgen treatments of bluegill. Journal of Applied Ichthyology 18, 61–64.

Ali, M., Nicieza, A., Wootton, R.J., 2003. Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4, 147–190.

Arslan, T., 2002. Sex determination in selected Centrarchids. Ph.D. dissertation. Auburn University, Auburn, AL. pp 13–161.

Arslan, T., Phelps, R.P., 2004. Directing gonadal differentiation in bluegill, *Lepomis marochirus* (Rafinesque), and black crappie, *Pomoxis nigromaculatus* (Lesueur), by periodic estradiol-17β immersions. Aquaculture Research 35, 397–402.

Bennett, G.W., 1971. Management of Lakes and Ponds. Van Nostrand Reinhold Co., New York, New York, USA.

Blázquez, M., Zanuy, S., Carrillo, M., Piferrer, F., 1998. Structural and functional effects of early exposure to estradiol-17β and 17a-ethynylestradiol on the gonads of the gonochoristic teleost *Dicentrarchus labrax*. Fish Physiology and Biochemistry 18, 37–47.

Brunson, M.W., Robinette, H.R., 1986. Evaluation of male bluegill × female green sunfish hybrids for stocking in Mississippi farm ponds. North American Journal of Fisheries Management 6, 156–167.

Brunson, M.W., Morris, J.E., 2000. Species profile: sunfish. Southern Regional Aquaculture Center Publication No. 724, p. 6. Stoneville, Mississippi, MS.

Chevassus, B., Devaux, A., Chourrout, D., Jalabert, B., 1988. Production of YY rainbow trout males by self-fertilization of induced hermaphrodites. Journal of Heredity 79, 89–92.

Chew, L.E., Stanley, J.G., 1973. The effect of methyltestosterone on sex reversal in bluegill. The Progressive Fish-Culturist 35, 44–47.

Clark Jr., R.D., Lockwood, R.N., 1990. Population dynamics of bluegills subjected to harvest within the 5.0- to 6.9-inch size range. Michigan Department of Natural Resources, Fisheries Research Report 1961, Ann Arbor.

Donaldson, E.M., Hunter, G.A., 1982. Sex control in fish with particular reference to salmonids. Canadian Journal of Fisheries and Aquatic Sciences 39, 99–110.

Dunham, R.A., 1990. Production and use of monosex and sterile fishes in aquaculture. Reviews in Aquatic Sciences 2, 1–17.

Ehlinger, T., 1989. Learning and individual variation in bluegill foraging: habitat-specific techniques. Animal Behavior 38, 643–658.

Emig, J.W., 1966. Largemouth bass. In: Calhoun, A. (Ed.), Inland Fisheries Management. California Department of Fish and Game, Sacramento, California, USA, pp. 332–353.
Fagerlund, U.H.M., McBride, J.R., 1975. Growth increments and some flesh and gonad characteristics of juvenile coho salmon receiving diets supplemented with 17a-methyltestosterone. Journal of Fish Biology 7, 305–314.
Fields, J., Pierskalla, C., Schuett, M., DeCarlo, K., 2004. Determining Demand and Describing a New Market Segment: Hybrid Bluegill and Recreational Pay Fishing. West Virginia University. http://www.wvu.edu/~agexten/aquaculture/2004forum/Fields.pdf.
Funk, J.D., Donaldson, E.M., Dye, H.M., 1973. Induction of precocious sexual development in female pink salmon (*Oncorhynchus gorbuscha*). Canadian Journal of Zoology-Revue Canadienne De Zoologie 51, 493–500.
George, T., Pandian, T.J., 1996. Hormonal induction of sex reversal and progeny testing in the zebra cichlid *Cichlasoma nigrofasciatum*. Journal of Experimental Zoology 275, 374–382.
George, T., Pandian, T.J., 1998. Dietary administration of androgens induces sterility in the female-heterogametic black molly, *Poecilia sphenops* (Cuvier & Valenciennes, 1846). Aquaculture Research 29, 167–175.
Goetz, F.W., Donaldson, E.M., Hunter, G.A., 1979. Effects of estradiol-17β and 17a-methyltestosterone on gonadal differentiation in the coho salmon, *Oncorhynchus kisutch*. Aquaculture 17, 267–278.
Guerrero, R.D., Shelton, W.L., 1974. An aceto-carmine squash method of sexing juvenile fishes. The Progressive Fish-Culturist 36, 56.
Hayward, R.S., Wang, H.P., 2002. Inherent growth capacity and social cost in bluegill and hybrids of bluegill and green sunfish: which fish really grows faster. North American Journal of Aquaculture 64, 34–46.
Hayward, R.S., Wang, H.P., 2006. Rearing male bluegills indoors may be advantageous for producing food-size sunfish. Journal of the World Aquaculture Society 37, 496–508.
Hunter, G.A., Donaldson, E.M., 1983. Hormonal sex control and its application to fish culture. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology, vol. 9B. Academic Press, New York, NY, USA, pp. 223–291.
Hunter, G.A., Solar, I.I., Baker, I.J., Donaldson, E.M., 1986. Feminization of coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) by immersion of alevins in a solution of estradiol-17β. Aquaculture 53, 295–302.
Johnstone, R., Simpson, T.H., Youngson, A.F., 1978. Sex reversal in salmonid culture. Aquaculture 13, 115–134.
Johnstone, R., Simpson, T.H., Youngson, A.F., 1979. Sex reversal in salmonid culture. Part II. The progeny of sex-reversed rainbow trout. Aquaculture 18, 13–19.
Kavumpurath, S., Pandian, T.J., 1992a. Production of YY male in the guppy, *Poecilia reticulata*, by endocrine sex reversal and progeny testing. Asian Fisheries Society 5, 265–276.
Kavumpurath, S., Pandian, T.J., 1992b. The development of all-male sterile triploid fighting fish (*Betta splendens*-set manipulation. Israeli Journal of Aquaculture-Bamidgeh 44, 111–119.
Kim, D.S., Nam, Y.K., Jo, J.Y., 1997. Effect of oestradiol-17β immersion treatments on sex reversal of mud loach, *Misgurnus mizolepis*. Aquaculture Research 28, 941–946.
Kohler, C.C., Kelly, A.M., 1991. Assessing predator-prey balance in impoundments. Proceedings of the Warmwater Fisheries Symposium I, June 4–8, 1991, Scottsdale, Arizona. USDA Forest Service, General Technical Report RM-207, pp. 257–260.
Lewis, W.M., Heidinger, R.C., 1978. Use of hybrids in the management of small impoundments. In: Novinger, G.D., Dillard, J.C. (Eds.), New Approaches for the Management of Small Impoundments North Central Division. American Fisheries Society, Soecial Publication 5, Bethesda, Maryland, pp. 104–108.
Liu, S., Yao, Z., Wang, Y., 1996. Sex hormone induction of sex reversal in the teleost *Clarias lazera* and evidence for female homogamety and male heterogamety. Journal of Experimental Zoology 276, 432–438.
Lutz, G., Sambidi, P., Harrison, R., 2003. Tilapia Industry Profile. Agriculture Marketing Resource Center. Iowa State University. http://test.agmrc.org/ aquaculture/profiles/tilapiaprofile.pdf.
Malison, I.A., Kayes, T.B., Wentworth, B.C., Amundson, C.H., 1988. Growth and feeding responses of male versus female yellow perch (*Perca flavescens*) treated with estradiol-17β. Canadian Journal of Fisheries and Aquatic Science 45, 1942–1948.
McBride, J.R., Fagerlund, U.H.M., 1973. The use of 17a-methyltestosterone for promoting weight increases in juvenile Pacific salmon. Journal of the Fisheries Research Board of Canada 30, 1099–1104.
McLarney, W., 1987. Characteristics of important cultured animals summarized. The Freshwater Aquaculture Book. Hartley & Marks, Point Roberts, Washington, pp. 485–508.
Mires, D., 1995. The tilapias. In: Nash, C.E., Novotony, A.J. (eds.) Production of Aquatic Animals, Chap. 7. Elsevier, New York, NY, USA, pp. 133–152NCRAC. 2002. Annual Report of the North Central Regional Aquaculture Center. Michigan State University, E. Lansing.
Mitzner, L., 1984. Crappie management: problems and solutions. North American Journal of Fisheries Management 4, 339–340.
Mommsen, T.P., Walsh, P.J., 1988. Vitellogenesis and oocyte assembly. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol 11A. Academic, New York, pp. 347–406.
Onozato, H., 1993. Direct production of the super male (YY) by androgenesis in the amago salmon. Biology International, Special Issue 28, 69–71.
Pandian, T.J., Sheela, S.G., 1995. Hormonal induction of sex reversal in fish. Aquaculture 138, 1–22.
Park, I.S., Kim, J.H., Bang, I.C., Kim, D.S., 1998. Histological study of the early gonadal development and sexual differentiation in *Rhynchocypris oxycephalus*. Development and Reproduction 2, 69–74.
Park, I.S., Kim, J.H., Cho, S.H., Kim, D.S., 2004. Sex differentiation and hormonal sex reversal in the bagrid catfish *Pseudobagrus fulvidraco* (Richardson). Aquaulture 232, 183–193.
Piferrer, F., 2001. Endocrine sex control strategies for the feminization of teleost fish. Aquaculture 197, 229–281.
Piferrer, F., Donaldson, E.M., 1992. The comparative effectiveness of the natural and a synthetic estrogen for the direct feminization of chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 106, 183–193.
Satoh, H., Nimura, Y., 1991. Growth promotion in Japanese eel by the oral administration of an estrogen (diethylstilbestrol). Nippon Suisan Gakkaishi 57, 21–27.
Schmittou, H.R., 1967. Sex ratios of bluegill in four populations. Transactions of the American Fisheries Society 96, 420–421.
Schreck, C.B., 1974. Hormonal treatment and sex manipulation in fishes. In: Schreck, C.B. (Ed.), Control of Sex in Fishes vol. VPI-SG-74-01. Extension Division, Virginia Polytechnical Institute and State University, Blacksburg, pp. 84–106.
Scott, A.G., Penman, D.J., Beardmore, J.A., Skibinski, D.O.F., 1989. The 'YY' supermale in *Oreochromis niloticus* (L.) and its potential in aquaculture. Aquaculture 78, 237–251.
Tave, D., 1993. Genetics for Fish Hatchery Managers, (2nd edition). Van Nostrand Reinhold, New York.
Tidwell, J.H., Webster, C.D., 1993. Effects of stocking density and dietary protein on green sunfish (*Lepomis cyanellus*) × bluegill (*L. macrochirus*) hybrids overwintered in ponds. Aquaculture 113, 83–89.
Varadaraj, K., Pandian, T.J., 1989. First report on production of supermale tilapia by integrating endocrine sex reversal with gynogenetic technique. Current Science 58, 434–441.
Wang, H.P., Wallat, G.K., Hayward, R., Tiu, L.G., O'Bryant, P., and Rapp, D., in press. Establishment of mostly-male groups of bluegill by grading selection and evaluation of their growth performance. North American Journal of Aquaculture.
Woo, N.Y.S., Chung, A.S.B., Ng, T.B., 1993. Influence of oral administration of estradiol-17β and testosterone on growth, digestion, food conversion and metabolism in the underyearling red sea bream, *Chrysophrys major*. Fish Physiology and Biochemistry 10, 377–387.
Yamamoto, T., 1964. The problem of viability of YY zygotes in the medaka, *Oryzias latipes*. Genetics 50, 45–58.
Yamamoto, T., 1975. A YY male goldfish from mating estrone-induced XY female and normal male. Journal of Heredity 66, 2–4.
Yamazaki, F., 1983. Sex control and manipulation in fish. Aquaculture 33, 329–354.