Mar Biotechnol (2009) 11:210–222
DOI 10.1007/s10126-008-9136-3

ORIGINAL ARTICLE

Seasonal and Sex-specific mRNA Levels of Key Endocrine Genes in Adult Yellow Perch (*Perca flavescens*) from Lake Erie

S. G. Lynn · K. A. Powell · D. F. Westneat · B. S. Shepherd

Received: 24 April 2008 / Accepted: 27 July 2008 / Published online: 23 September 2008

Abstract To better understand the endocrine mechanisms that underlie sexually dimorphic growth (females grow faster) in yellow perch (*Perca flavescens*), real-time quantitative polymerase chain reaction (qPCR) was used to measure pituitary, liver, and ovary mRNA levels of genes related to growth and reproduction–sex in this species. Adult perch were collected from Lake Erie and body mass, age, gonadosomatic index (I_G), hepatosomatic index (I_H), and gene expression for growth hormone (GH), prolactin, somatolactin, insulin-like growth factor Ib (IGF-Ib), estrogen receptor α (*esr1*), estrogen receptor βa (*esr2a*), and aromatase (*cyp19a1a*) were measured. Females had higher body mass, I_H, and liver *esr1* mRNA level than males, while males had higher liver IGF-Ib, liver *esr2a*, and liver *cyp19a1a* mRNA levels. In both sexes, season had a significant effect on GH and liver IGF-Ib mRNAs with higher levels occurring in spring, which also corresponded with higher liver *cyp19a1a* mRNA levels. For females, I_G, liver *esr1*, and ovary *cyp19a1a* mRNA levels were higher in autumn than the spring, and ovary *cyp19a1a* mRNA levels showed a significant negative correlation with pituitary GH and liver IGF-Ib mRNA levels. The most significant ($p \leq 0.001$) relationships across the parameters measured were positive correlations between liver IGF-Ib and *esr2a* mRNA levels and liver IGF-Ib and *cyp19a1a* mRNA levels. This study shows significant effects of season and sex on adult yellow perch endocrine physiology.

Keywords Yellow perch · Hormone · Physiology · mRNA · Teleost

S. G. Lynn · D. F. Westneat
Department of Biology, University of Kentucky,
Lexington, KY 40506-0225, USA

S. G. Lynn
e-mail: sglynn@msu.edu

K. A. Powell
49 Cummings Avenue,
Buxton, ME 04093, USA

B. S. Shepherd (✉)
Great Lakes WATER Institute/ARS/USDA,
600 East Greenfield Avenue,
Milwaukee, WI 53204, USA
e-mail: brian.shepherd@ars.usda.gov

Present Address:
S. G. Lynn
Michigan State University,
224 Biochemistry Building,
East Lansing, MI 48824-1319, USA

Introduction

Yellow perch (*Perca flavescens* Mitchell) is an economically, ecologically, and recreationally important teleost species throughout the upper Midwest (Malison 1999), especially in the Great Lakes, and have historically comprised the largest inland fishery in North America (Troutman 1980). Commercial fisheries harvest more than ten million pounds per year of yellow perch from the Great Lakes, with greater than 80% coming from Lake Erie (Malison 1999; Kinnunen 2003). Yellow perch are one of a group of fishes (including flatfish, carp, and salmon, etc.) which exhibit a sexual size dimorphism (SSD) in which females grow faster than males (Malison et al. 1988; Devlin and Nagahama 2002; Chen et al. 2007). The basis for SSD is complex and unclear, but growth in species that display SSD has been linked to hormonal cues involved with sexual maturation (Devlin and Nagahama 2002). As for yellow perch, the female-biased SSD has been demonstrated in

laboratory (Schott et al. 1978) and wild populations (Hile and Jobes 1942), but it was not until the mid-1980s that 17β-estradiol (E_2) was determined to be a growth promoter in yellow perch SSD (Malison et al. 1985, 1986, 1988). In a series of experiments using E_2, MT, triiodothyronine, and zeranol, with different size classes of juvenile perch, Malison et al. (1985) found that only the low dosages of E_2 (2 and 20 μg g^{-1} diet) significantly stimulated growth in both sexes of fish which were 80–110 mm total length (TL) or greater. The critical size range of 80–110-mm TL is the same size at which females normally begin to outgrow males (Schott et al. 1978) and female-biased SSD is first observed. This size range is also the minimum body size for the onset of vitellogenesis in females and spermatogenesis in males (Malison et al. 1986), suggesting an upregulation of E_2 receptors (ERs) on target tissues (ovary, liver, or pituitary) and a coinciding increase in tissue expression of growth factors. While yellow perch SSD is first manifested in juvenile fish (80–110-mm TL), it remains readily apparent in adult yellow perch with or without estrogen treatment. Hayward and Wang (2001) found that growth rate and growth efficiency of adult female yellow perch exceeded those of males twofold in animals fed without restriction or hormone treatment. These observations point out a clear linkage between growth and reproductive endocrinology in this species. Similar relationship have been shown in salmonids and the sex-dependent growth has been linked to chromosomal regions associated with sex determination, sexual maturation, and reproductive hormones known to be involved with steroid production (Devlin and Nagahama 2002; Haidle et al. 2008; Patil and Hinze 2008).

Several endocrine genes associated with growth and estrogen physiology have recently been cloned and characterized in the yellow perch (Roberts et al. 2004; Lynn and Shepherd 2007; Lynn et al. 2008). In vertebrates, the pituitary gland is the "master gland" and as such at least partially regulates aspects of growth and reproduction. Pituitary growth hormone (GH) and insulin-like growth factor I (IGF-I), primarily produced in the liver, are considered to be the key players in the growth process (Duan 1997). Many studies have shown a causative effect of GH on the release of hepatic IGF-I (Carnevali et al. 2005) and it is believed that much of the effect GH has on growth is mediated through IGF-I (Vong et al. 2003). There is increasing evidence that somatolactin (SL), a pituitary hormone found only in fish, is involved in metabolism, sexual maturation, and reproductive cycle regulation (Mayer et al. 1998) with the highest SL receptor levels in masu salmon (*Oncorhynchus masou*) found in liver and fat tissue (Fukada et al. 2005). Prolactin (PRL), another pituitary hormone, has long been suspected of involvement in fish reproduction based on its well-known role in mammalian reproduction (Freeman et al. 2000) and the ability of tilapia (*Oreochromis mossambicus* Peters) PRL ($tPRL_{177}$) to elevate IGF-I mRNA levels in the liver (Shepherd et al. 1997) indicates that PRL may possess somatotropic actions similar to GH. Estrogen receptors (ERs) are distributed in many tissues in teleosts (gonad, liver, brain, and pituitaries; Choi and Habibi 2003; Lynn et al. 2008) and are intricately involved in sexual determination and development (Guiguen et al. 1999). Estrogen levels, however, are controlled primarily by P450 aromatase (*cyp19a1a*), the terminal enzyme in the conversion of testosterone to estrogen. *Cyp19a1a* is expressed in many tissues associated with reproduction (gonad, brain, and pituitary; Kumar et al. 2000; Lynn et al. 2008) and growth (liver, brain, and pituitary; Menuet et al. 2003; Lynn et al. 2008) and varies with sexual maturation (Melo and Ramsdell 2001), age, and season (González and Piferrer 2003). Because of the clear link between growth and sex in yellow perch, there is a need for a better understanding of the basal levels and interactions of these endocrine genes in this species.

The cDNAs for yellow perch GH, PRL, SL, IGF-Ib, *esr1*, *esr2a*, and *cyp19a1a* have recently been published (Roberts et al. 2004; Lynn and Shepherd 2007; Lynn et al. 2008) and in this study we developed real-time quantitative PCR (qPCR) assays to measure mRNA or expression levels of these genes in a natural population of yellow perch from Lake Erie. Against this background, yellow perch were sampled twice per year, just after spawning (May) and during the autumnal period when growth and gonadal recrudescence occurs (October), over a 2-year period. We examined the seasonal expression levels of these key endocrine genes in target tissues, from both adult male and female yellow perch, to obtain baseline data on the seasonal expression of these genes to gain a better understanding of their sex-specific regulation which will hopefully aid in future efforts aimed at elucidating the potential endocrine pathways involved with estrogen-stimulated SSD in this species.

Materials and Methods

Sample Collections

Adult yellow perch (*P. flavescens*) from Lake Erie were sampled in late May and October for 2 years from October 2002 to May 2004. Trap nets owned by a commercial fisheries company, Swartz Fisheries (Port Clinton, OH, USA), which are designed to exclude fish less than 15.24 cm (6 in.) in length, were set (~12-m depth) and maintained just north of Sandusky, OH, USA (approximate N 41 22 875, W 082 30 833) for the duration of the yellow perch commercial fishing season (May to October) and were

tended every 3 days. On sample days, nets were pulled and up to 30 adult fish were randomly placed into 19-L aerated buckets (five per bucket). Fish were anesthetized with 50 mg L^{-1} of MS222 and 100 mg L^{-1} of $NaHCO_3$ to minimize a stress response and, once on shore, the fish were given a lethal dose of MS222 (1 g L^{-1}), weighed, and sexed and pituitaries, livers, and ovaries were collected. Whole livers and ovaries were weighed to determine hepatosomatic index (I_H) and gonadosomatic index (I_G), respectively, and tissues were immediately frozen, transported to the University of Kentucky, and stored at −80°C until analysis.

Sample Preparations and Analysis

Otoliths were removed and age was determined by drying, viewing a fractured portion of the otolith under a dissecting microscope and counting the annular rings (DeVries and Frie 1996). Age was not determined for all fish, as in some cases otoliths were not recovered. For each sampling time point (four: twice per year for 2 years), six male and six female sets of samples were randomly chosen for gene expression analyses. Ovaries were stripped of oocytes and manually homogenized and approximately 1 mg of each ovary and liver tissue was mechanically homogenized for RNA extraction. Whole pituitaries were mechanically homogenized and total RNA was extracted from all samples with the GenElute™ Mammalian Total RNA Kit (Sigma, St. Louis, MO, USA) and treated with amplification-grade DNase I (Sigma, St. Louis, MO, USA). RNA samples were quantified on a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) and 750 ng of sample was reverse-transcribed to cDNA using iScript™ cDNA Synthesis Kit (BioRad, Hercules, CA, USA). cDNA samples were treated with amplification-grade RNase (Sigma, St. Louis, MO, USA) and quantified.

Real-Time qPCR

Sequences for yellow perch GH (*AY007303*), PRL (*AY332491*), SL (*AY332490*), IGF-Ib (*AY332492*), *esr1* (*DQ984124*), *esr2a* (*DQ984125*), and *cyp19a1a* (*DQ984126*) are available from the GenBank–EMBL–DDBJ nucleotide sequence database. Primers (Table 1) were designed for real-time qPCR using Beacon Designer v. 3.0 (PREMIER Biosoft International, Palo Alto, CA, USA). Designed primers were tested with a 25 μl total volume PCR mixture using a MasterTaq Kit (Eppendorf Scientific Inc., Westbury, NY, USA) and 1 μl of cDNA template. GH, PRL, and SL used pituitary tissue cDNA as a template; IGF-Ib used liver tissue cDNA as a template and *esr1*, *esr2a*, and *cyp19a1a* used gravid ovary tissue cDNA as a template. Real-time qPCR consisted of 3 min at 94°C follow by 45 cycles of 45 s at 94°C and 45 s at 60°C. For product size and identity verification, PCR products were electrophoresed in 1% low melt agarose–2% nuseive gels with a 100-bp DNA ladder (Takara Shuzo Co., Otsu, Japan) and visualized by ethidium bromide staining. PCR products were then purified using Amicon Centrifugal Ultrafiltration Devices (Millipore, Billerica, MA, USA) and quantified. Purified PCR products were ligated into a pCR®4-TOPO® vector and transformed into TOP10 chemically competent cells using the TOPO TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, CA, USA). The plasmid DNA was then extracted from the bacterial cells using the GenElute™ Plasmid Miniprep Kit (Sigma, Sigma, St. Louis, MO, USA). Plasmid samples were quantified and up to 600 ng of plasmid DNA was used for PCR sequencing using BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Products were sequenced at the University of Kentucky Advanced Genetic Technologies Center. Sequencing products were compared to known template

Table 1 Forward (F) and reverse (R) primer sequences and Accession numbers used for gene specific qPCR assays

Target Gene	Accession #		Start	Primer sequence
GH	AY007303	F	224	CGGAGGAGCAGCGTCAAC
		R	370	CCCAGGACTCGACCAAACG
PRL	AY332491	F	304	ACCAGGCTCTTCAAGTATCAG
		R	406	GTGTTAGCAGAGGTGGAGAG
SL	AY332490	F	321	CTCCAAAGGTGAAATCCAACAG
		R	449	TCAGGAGCGGCATCGTAG
IGF-Ib	AY332492	F	539	CGCAGGGCACAAAGTGGAC
		R	686	CCCAGTGTTGCCTCGACTTG
esr1	DQ984124	F	1072	AGGTGCTGATGATCGGGCTC
		R	1165	TCGCCTACGTTCCTGTCCAG
esr2a	DQ984125	F	1887	TCTGGACGCTGTGACGGAC
		R	1969	GGGCGAGGCGGGTGTAC
cyp19a1a	DQ984126	F	278	TCTGGGTTTGGGGCCACTTC
		R	427	ACCGCTGATGCTCTGCTGAG

sequences using Vector NTI Suite 7.0 (Informax, Inc., Frederick, MD, USA) and GeneDoc (Nicholas et al. 1997) to verify primer specificity.

Generally, qPCR assays employ one of two standard procedures: relative or absolute quantification. Relative quantification compares expression of the target gene with three or more reference genes; however, recent studies have shown that expression levels of housekeeping (reference) genes can vary considerably within a sample set (Dheda et al. 2005; Arukwe 2006; Sellars et al. 2007). Given the limited number of reference genes available in yellow perch at the time of this study and a lack of knowledge regarding their expression levels, we chose to utilize absolute qPCR (Bustin 2000; Sellars et al. 2007). Purified PCR products were serially diluted to generate six standards (10,000, 1,000, 100, 10, 1, and 0.1 fg μl^{-1}). Real-time qPCR reactions (25 µl) were prepared in 0.2-ml thin-wall 96-well plates (BioRad, Hercules, CA, USA) each containing the following components: 12.5 µl iQ™ SYBR® Green Supermix (BioRad, Hercules, CA, USA), 0.75 µl (15 ng) each forward and reverse primers, 1 µl template, and 10 µl ddH_20. After pipetting, the plates were sealed with BioRad iCycler iQ Optical Tape and spun at 2,200 rcf for 1 min. Amplification and detection of samples were performed with the BioRad iCycler Thermal Cycler and Optical Module (BioRad, Hercules, CA, USA). Each 96-well plate had duplicate wells of standards, no template, RNA template, and samples. Duplicate sample Ct values with a coefficient of variation >2% were rerun. When necessary, dilutions of template cDNAs were performed to ensure samples fell within the standard curve. All plates had standard curves with $R>0.98$ and PCR efficiencies between 85% and 110% with the exception of IGF-Ib which had PCR efficiencies up to 114%. Melt curves were performed following all runs to further verify primer template specificity. Real-time qPCR results (ag of template per microliter of cDNA) were standardized to cDNA concentration ($\mu g\ \mu l^{-1}$) and log-transformed before statistical analyses.

Statistics

I_H and I_G proportions were arcsine-transformed (arcsine of square root for each value; Sokal and Rohlf 1995) and tested for deviations from a normal distribution before any statistical analyses were performed. Statistical outliers within each variable were identified as data points beyond three times the interquartile range using a box plot. Only one data point, a GH mRNA measurement from an autumnal male, was defined as an outlier and removed from all analyses. A general linear model (GLM) was used to determine year effects for each variable and if a year effect existed the data were divided into sex-specific seasonal groups for further clarification of the year effect. Since only one variable showed a year effect, the data from both years were combined and a GLM was used to examine variables (body mass, I_H, and GH, PRL, SL, IGF-Ib, liver *esr1*, liver *esr2a*, and liver *cyp19a1a* mRNA levels) for sex, season, age, sex × season interaction, and sex × season × age interaction effects. Differences among groups were further evaluated by two-way analysis of variance (GLM procedure) with sex and season as independent variables. Pairwise comparisons between groups (e.g., autumn male, spring female, etc.) were performed using Tukey's post hoc pairwise comparison for variables (body mass, I_H, and GH, SL, IGF-Ib, liver *esr1*, liver *esr2a*, and liver *cyp19a1a* mRNA levels) which showed a significant ($p<0.05$) sex or season effect. For variables with only female data (I_G and ovary *esr1*, ovary *esr2a*, and ovary *cyp19a1a* mRNA levels), a GLM was used to examine for season, age, and season × age interaction effects only. For female-only variables which did not show an age or season × age interaction effect, a *t* test was performed to examine for season effects only. Relationships between 13 measured variables were determined by generating a Pearson (*r*) correlation matrix; significant differences from 0 were tested using Fisher's *z* (Zar 1999). SYSTAT Grad Pack v. 10.0 (Systat, Chicago, IL, USA) was used for all analyses and differences between groups and relationships between parameters were considered significant at $p\leq0.05$. Figures were generated to illustrate effects of season and sex (bar graphs), but when there was a significant effect of age or an age interaction (e.g., body mass, liver *cyp19a1a*, and ovary *esr1*) or a post hoc *t* test was significant (e.g., I_G and ovary *cyp19a1a*) a scatter plot figure was necessary.

Results

Year Effects

There was a significant effect of year on I_H ($F_{1,\ 46}=8.0$; $p=0.007$) only, but when the data were separated into sex-specific seasonal groups (e.g., autumn males, spring females, etc.) only the autumn groups (autumn males, $p=0.034$; autumn females, $p=0.004$) showed significant differences between years for I_H, with year 2 being higher than year 1, for both sexes.

Sex, Season, and Age Effects

There were significant effects of sex and age on body mass, with female yellow perch having a greater mean body mass (~176 g) than male (~144 g) yellow perch (Fig. 1). Female yellow perch showed a positive relationship between body mass and age with the older fish being larger than the

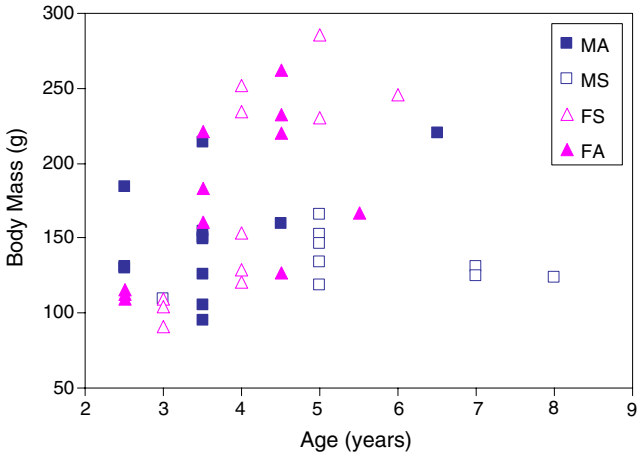

Fig. 1 Age vs. body mass for individual male (*M*) and female (*F*) Lake Erie yellow perch in autumn (*A*) and spring (*S*). Sample sizes for each sex-specific seasonal group were: MA=12, MS=9, FA=11 and FS=11. Body mass showed significant effects of sex (p=0.04), with females ($\overline{X}$=176±14) being heavier than males ($\overline{X}$=144±7), and age (p=0.001), with older fish generally being heavier, but no effect of season (p=0.11), nor sex × season (p=0.21) or sex × season × age (p=0.07) interactions. There were no significant differences at $p \leq 0.05$ between each sex-specific seasonal group (*MA*, *MS*, *FA*, and *FS*)

younger fish, whereas male body mass was not related to age. There was a significant effect of season on GH mRNA levels with spring yellow perch having higher GH mRNA levels than autumn yellow perch (Fig. 2). For pituitary PRL mRNA levels, there were no significant effects of sex ($F_{1,\ 37}$=0.04; p=0.84), season ($F_{1,\ 37}$=0.7; p=0.40), age ($F_{1,\ 37}$=0.8; p=0.38), sex × season interaction ($F_{1,\ 37}$=0.2; p=0.64), or sex × season × age interaction ($F_{1,\ 37}$=0.1; p=0.71; data not shown). Only season had a significant effect on SL mRNA levels with spring yellow perch having higher SL mRNA levels than autumn yellow perch (Fig. 3).

There was a significant effect of sex on I_H with female yellow perch having a higher average I_H than male yellow perch (Fig. 4). Both sex and season had significant effects on liver IGF-Ib mRNA levels with male yellow perch having higher liver IGF-Ib mRNA levels than female yellow perch and spring yellow perch having higher liver IGF-Ib mRNA levels than autumn yellow perch (Fig. 5). There were also significant effects of sex and season on liver *esr1* mRNA levels with female yellow perch having higher liver *esr1* mRNA levels than male yellow perch and autumn yellow perch having higher liver *esr1* mRNA levels than spring yellow perch (Fig. 6). There was a significant effect of sex on liver *esr2a* mRNA levels with male yellow perch having higher liver *esr2a* mRNA levels than female yellow perch (Fig. 7). While there were no significant effects of season or sex × season interaction on liver *esr2a* mRNA levels, levels in males were significantly greater than levels in females but only in autumn and not in spring (Fig. 7). There were significant effects of both sex and season on liver *cyp19a1a* mRNA levels with male yellow perch having higher liver *cyp19a1a* mRNA levels than female yellow perch and spring yellow perch having higher liver *cyp19a1a* mRNA levels than autumn yellow perch (Fig. 8). Also, there were significant effects of age and sex × season × age interaction on liver *cyp19a1a* (Fig. 8)

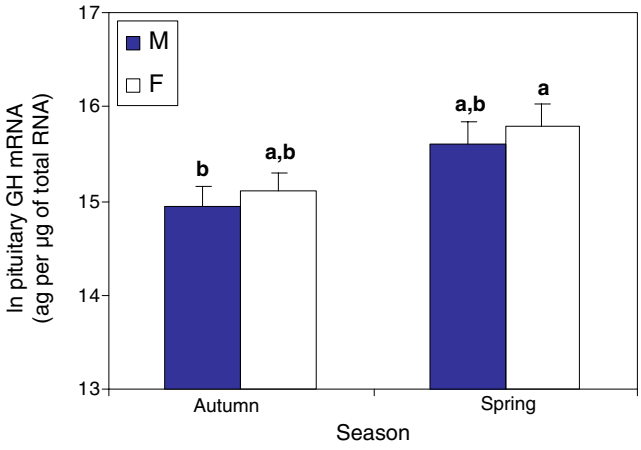

Fig. 2 Mean pituitary GH mRNA levels for male (*M*) and female (*F*) Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for GH are expressed as ln (ag of GH mRNA per microgram of total RNA). *Bars* indicate standard error and sample sizes for each sex-specific seasonal group were: MA=11, MS=9, FA=11, and FS=11. GH mRNA levels showed a significant effect of season (p=0.001), with spring ($\overline{X}$=15.70±0.16) being higher than autumn ($\overline{X}$=15.08±0.13), but no effect of sex (p=0.54), age (p=0.06), nor sex × season (p=0.59) or sex × season × age (p=0.47) interactions. Groups with different alpha characters (*a–b*) are significantly different at $p \leq 0.05$ from other groups

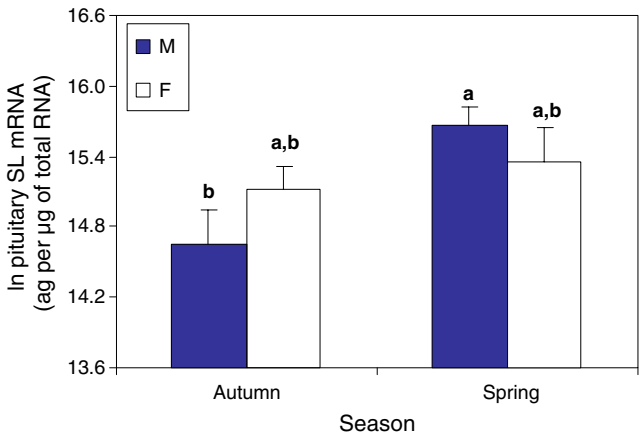

Fig. 3 Mean pituitary SL mRNA levels for male (*M*) and female (*F*) Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for SL are expressed as ln (ag of SL mRNA per microgram of total RNA). *Bars* indicate standard error and sample sizes for each sex-specific seasonal group were: MA=12, MS=9, FA=11, and FS=11. SL mRNA levels showed a significant effect of season (p=0.02), with spring ($\overline{X}$=15.49±0.17) being higher than autumn ($\overline{X}$=14.89±0.19), but no effect of sex (p=0.66), age (p=0.42), nor sex × season (p=0.79) or sex × season × age (p=0.46) interactions. Groups with different alpha characters (*a–b*) are significantly different at $p \leq 0.05$ from other groups

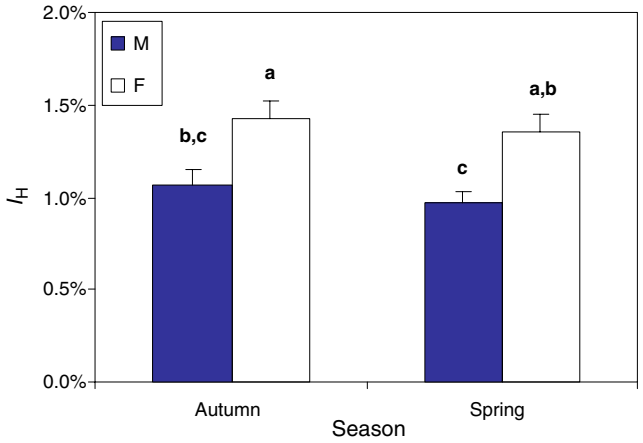

Fig. 4 Mean I_H for male (*M*) and female (*F*) Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for I_H (hepatosomatic index) are expressed as percentage liver mass of body mass. *Bars* indicate standard error and sample sizes for each sex-specific seasonal group were: MA=12, MS=9, FA=11, and FS=11. I_H showed a significant effect of sex (p=0.002), with females ($\overline{X}$=1.39±0.06) being higher than males ($\overline{X}$=1.03±0.05), but no effect of season (p=0.48), age (p=0.61), the sex × season interaction (p=0.08) nor the sex × season × age interaction (p=0.07). Groups with different alpha characters (*a*–*c*) are significantly different at $p \leq 0.05$ from other groups

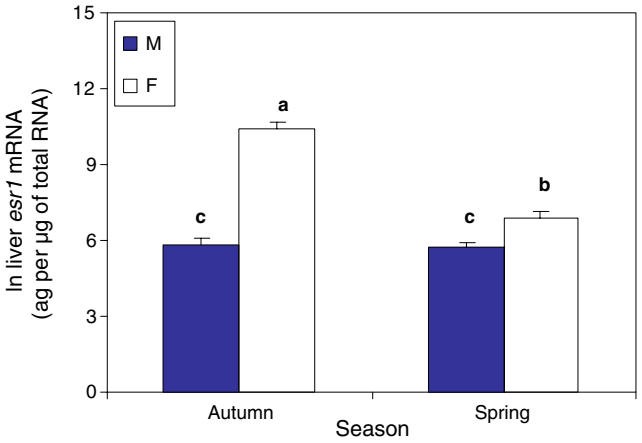

Fig. 6 Mean liver *esr1* mRNA levels for male (*M*) and female (*F*) Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for liver *esr1* are expressed as ln (ag of liver *esr1* mRNA per microgram of total RNA). *Bars* indicate standard error and sample sizes for each sex-specific seasonal group were: MA=12, MS=9, FA=11, and FS=11. Liver *esr1* mRNA levels showed significant effects of sex (p<0.001), with females ($\overline{X}$=8.65±0.43) being higher than males ($\overline{X}$=5.78±0.15), and season (p<0.001), with autumn ($\overline{X}$=8.11±0.54) being higher than spring ($\overline{X}$=6.36±0.22), but no effect of age (p=0.98), the sex × season interaction (p=0.21) nor the sex × season × age interaction (p=0.65). Groups with different alpha characters (*a*–*c*) are significantly different at $p \leq 0.05$ from other groups

with a general trend of higher liver *cyp19a1a* mRNA levels in younger yellow perch.

There were no significant effects of season, age nor season × age interaction on I_G in the yellow perch sampled (Fig. 9). However, because the data from Fig. 9 suggest a seasonal difference, a *t* test was performed to compare spring and autumn I_G in female yellow perch. The *t* test reveals that autumn females, with ovaries containing

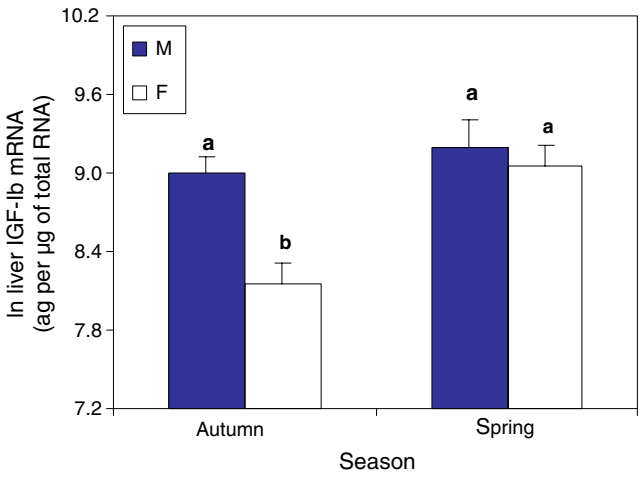

Fig. 5 Liver IGF-Ib mRNA levels for male (*M*) and female (*F*) Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for IGF-Ib are expressed as ln (ag of IGF-Ib mRNA per microgram of total RNA). *Bars* indicate standard error and sample sizes for each sex-specific seasonal group were: MA=12, MS=9, FA=11, and FS=11. Liver IGF-Ib mRNA levels showed significant effects of sex (p=0.004), with males ($\overline{X}$=9.09±0.11) being higher than females ($\overline{X}$=8.61±0.14), and season (p<0.001), with spring ($\overline{X}$=9.12±0.11) being higher than autumn ($\overline{X}$=8.59±0.14), but no effect of age (p=0.14), the sex × season interaction (p=0.40) nor the sex × season × age interaction (p=0.68). Groups with different alpha characters (*a*–*b*) are significantly different at $p \leq 0.05$ from other groups

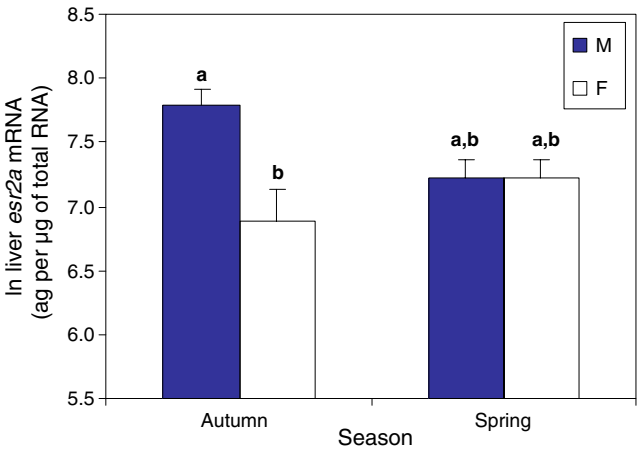

Fig. 7 Mean liver *esr2a* mRNA levels for male (*M*) and female (*F*) Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for liver *esr2a* are expressed as ln (ag of liver *esr2a* mRNA per microgram of total RNA). *Bars* indicate standard error and sample sizes for each sex-specific seasonal group were: MA=12, MS=9, FA=11, and FS=11. Liver *esr2a* mRNA levels showed a significant effect of sex (p=0.02), with males ($\overline{X}$=7.54±0.11) being higher than females ($\overline{X}$=7.05±0.15), but no effect of season (p=0.68), age (p=0.88), the sex × season interaction (p=0.74) nor the sex × season × age interaction (p=0.78). Groups with different alpha characters (*a*–*b*) are significantly different at $p \leq 0.05$ from other groups

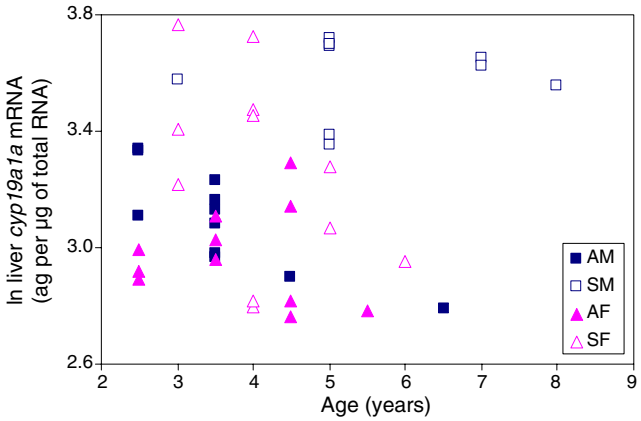

Fig. 8 Age vs. liver *cyp19a1a* mRNA levels for individual adult male (*M*) and female (*F*) Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for liver *cyp19a1a* are expressed as ln (ag of liver *cyp19a1a* mRNA per microgram of total RNA) and sample sizes for each sex-specific seasonal group were: MA=12, MS=9, FA=11, and FS=11. Liver *cyp19a1a* mRNA levels showed significant effects of sex (p=0.01), with males ($\overline{X}$=3.34±0.07) being higher than females ($\overline{X}$=3.12±0.07), season (p<0.001), with spring ($\overline{X}$=3.41±0.07) being higher than autumn ($\overline{X}$=3.08±0.05), age (p=0.04), with older fish generally being lower, and sex × season × age interaction (p=0.04), but no sex × season interaction (p=0.11). Pairwise comparisons for the following groups are indicated by superscript alpha characters (*a*–*c*) and groups with different *letters* are significantly significant at $p \leq 0.05$: MS^{a} ($\overline{X}$=3.58±0.05), FS^{b} ($\overline{X}$=3.27±0.11), MA^{b,c} ($\overline{X}$=3.16±0.09) and FA^{c} ($\overline{X}$=2.97±0.05)

previtellogenic oocytes, had a significantly higher average I_G than spring females, with postspawning ovaries that were deplete of all forms of gametes (S Lynn, personal observation). There was a significant effect of season on ovary *esr1* mRNA levels with spring yellow perch having higher ovary *esr1* mRNA levels than autumn yellow perch (Fig. 10) and there was also a significant season × age interaction on ovarian *esr1* mRNA levels. Ovary *esr2a* mRNA levels did not show a significant effect of season ($F_{1,\ 18}$=2.2; p=0.16), age ($F_{1,\ 18}$=0.3; p=0.60), or season × age interaction ($F_{1,\ 18}$=2.2; p=0.16; data not shown). There were no significant effects of season, age, nor season × age interaction on ovary *cyp19a1a* mRNA levels (Fig. 11). However, a *t* test revealed the ovary *cyp19a1a* mRNA levels were significantly higher in female yellow perch sampled in the autumn versus females sampled in the spring.

Correlations

A Pearson (*r*) correlation matrix for relationships between 13 measured variables is shown in Table 2. Body mass showed significant negative correlations with liver IGF-Ib and liver *cyp19a1a* mRNA levels. Pituitary GH mRNA levels showed significant positive correlations with PRL and SL mRNA levels and significant negative correlations with I_G and ovary *cyp19a1a* mRNA levels. Pituitary SL mRNA levels showed significant positive correlations with GH and liver *cyp19a1a* mRNA levels and a significant negative correlation with liver *esr2a* mRNA levels. I_H showed a significant positive correlation with liver *esr1* mRNA levels and significant negative correlations with IGF-Ib, liver *esr2a*, and liver *cyp19a1a* mRNA levels.

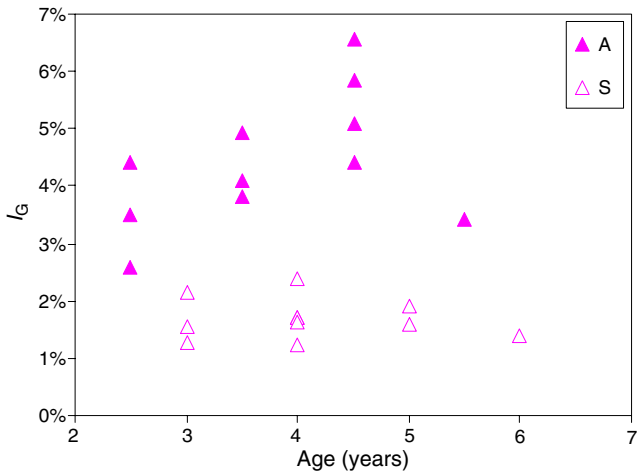

Fig. 9 Age vs. I_G for individual female Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for I_G (gonadosomatic index) are expressed as percentage ovary mass of body mass and sample sizes for each seasonal group were: *A*=11 and *S*=11. I_G showed no significant effect of season (p=0.48), age (p=0.23), or season × age interaction (p=0.16). A *t* test revealed a significant effect of season on I_G (p<0.001), with autumn levels ($\overline{X}$=4.43±0.36) being higher than spring levels ($\overline{X}$=1.68±0.11)

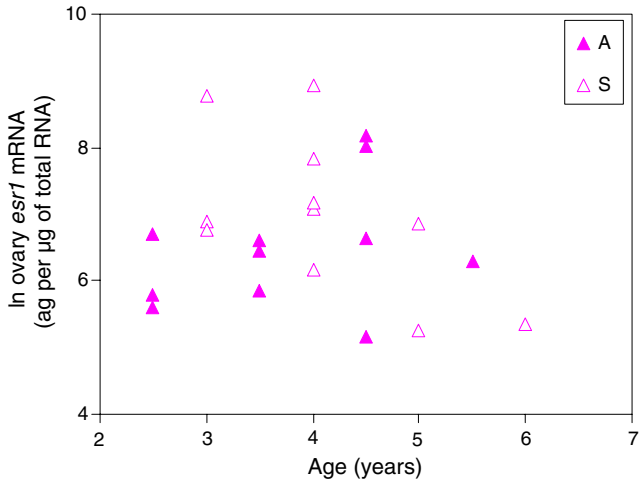

Fig. 10 Age vs. ovary *esr1* mRNA levels for individual female Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for ovary *esr1* are expressed as ln (ag of ovary *esr1* mRNA per microgram of total RNA) and sample sizes for each seasonal group were: *A*=11 and *S*=11. Ovary liver *esr1* mRNA levels showed significant effects of season (p=0.02), with spring levels ($\overline{X}$=7.01±0.38) being higher than autumn levels ($\overline{X}$=6.48±0.30), and season × age interaction (p=0.03), but no effect of age (p=0.33)

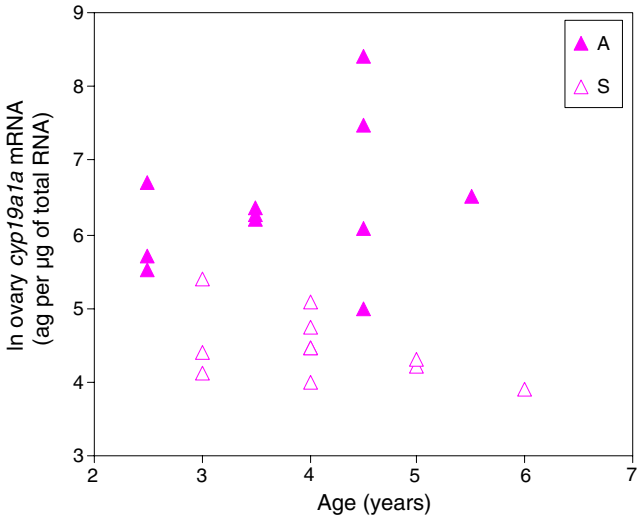

Fig. 11 Age vs. ovary *cyp19a1a* mRNA levels for individual female Lake Erie yellow perch in autumn (*A*) and spring (*S*). Units for ovary *cyp19a1a* are expressed as ln (ag of ovary *cyp19a1a* mRNA per microgram of total RNA) and sample sizes for each seasonal group were: *A*=11 and *S*=11. Ovary *cyp19a1a* mRNA levels showed no significant effect of season (p=0.96), age (p=0.86) or season × age interaction (p=0.14). A *t* test reveals a significant effect of season on ovary *cyp19a1a* mRNA levels (p<0.001), with autumn levels ($\overline{X}$= 6.38±0.29) being higher than spring levels ($\overline{X}$=4.47±0.14)

Liver IGF-Ib mRNA levels showed significant positive correlations with liver *esr2a* and liver *cyp19a1a* mRNA levels and significant negative correlations with body mass, I_H, I_G, liver *esr1*, and ovary *cyp19a1a* mRNA levels. Liver *esr1* mRNA levels showed significant positive correlations with I_H, I_G, and ovary *cyp19a1a* mRNA levels and significant negative correlations with liver IGF-Ib and liver *cyp19a1a* mRNA levels. Liver *esr2a* showed a significant positive correlation with IGF-Ib mRNA levels and significant negative correlations with I_H and SL mRNA levels. Liver *cyp19a1a* mRNA levels showed significant positive correlations with SL and IGF-Ib mRNA levels and significant negative correlations with body mass, I_H, I_G and liver *esr1* and ovary *cyp19a1a* mRNA levels. I_G showed significant positive correlations with liver *esr1* and ovary *cyp19a1a* mRNA levels and significant negative correlations with GH, IGF-Ib, and liver *cyp19a1a* mRNA levels. Ovary *esr1* mRNA levels showed a significant positive correlation only with ovary *esr2a* mRNA levels and vice versa. And finally, ovary *cyp19a1a* mRNA levels showed significant positive correlations with I_G and liver *esr1* mRNA levels and significant negative correlations with GH, IGF-Ib, and liver *cyp19a1a* mRNA levels.

Table 2 Pearson correlation matrix for 13 measured variables from adult Lake Erie yellow perch

n=48		Wt	GH[a]	PRL	SL	I_H	IGF-Ib	liver *esr1*	liver *esr2a*	liver *cyp19*	I_G[b]	ovary *esr1*[b]	ovary *esr2a*[b]
GH[a]	r	-0.09											
	p	0.55											
PRL	r	-0.04	0.45										
	p	0.77	0.002										
SL	r	-0.04	0.60	0.26									
	p	0.81	<0.001	0.07									
I_H	r	n/a	-0.06	-0.07	-0.05								
	p		0.69	0.65	0.72								
IGF-Ib	r	-0.33	0.12	-0.22	-0.01	-0.31							
	p	0.02	0.40	0.13	0.93	0.03							
liver *esr1*	r	0.13	-0.13	0.21	0.06	0.41	-0.46						
	p	0.37	0.37	0.15	0.71	0.003	0.001						
liver *esr2a*	r	-0.25	-0.27	-0.17	-0.31	-0.34	0.63	-0.16					
	p	0.08	0.07	0.26	0.03	0.02	<0.001	0.26					
liver *cyp19a1a*	r	-0.34	0.16	-0.08	0.35	-0.30	0.50	-0.35	0.14				
	p	0.02	0.28	0.61	0.02	0.04	<0.001	0.02	0.33				
I_G[b]	r	n/a	-0.40	0.21	0.04	0.26	-0.82	0.79	-0.38	-0.48			
	p		0.05	0.32	0.85	0.21	<0.001	<0.001	0.06	0.02			
ovary *esr1*[b]	r	-0.10	0.11	-0.05	0.07	0.10	0.15	-0.34	-0.18	0.34	-0.19		
	p	0.63	0.60	0.83	0.73	0.63	0.49	0.10	0.41	0.10	0.38		
ovary *esr2a*[b]	r	-0.01	-0.02	0.04	-0.11	-0.03	-0.01	-0.13	-0.12	0.12	-0.05	0.79	
	p	0.95	0.94	0.86	0.60	0.88	0.96	0.56	0.59	0.57	0.82	<0.001	
ovary *cyp19a1a*[b]	r	0.06	-0.49	0.18	-0.17	0.18	-0.59	0.72	-0.25	-0.49	0.75	0.23	0.39
	p	0.77	0.02	0.41	0.43	0.40	0.002	<0.001	0.23	0.02	<0.001	0.28	0.06

[a]single outlier removed, n=47; [b]female specific variable, n=24.

Discussion

This study reports a comprehensive measurement of the mRNA levels for key endocrine genes in a natural adult population of yellow perch from Lake Erie. The females sampled from this population were, on average, heavier than the males, had larger livers (I_H), lower liver IGF-Ib, liver *esr2a*, and liver *cyp19a1a* mRNA levels and higher liver *esr1* mRNA levels. Also, season had an effect on GH, SL, liver IGF-Ib, liver *esr1*, liver *cyp19a1a*, and ovary *esr1* mRNA levels. The lowest age in this study (2.5 years), which was above the average age for sexual maturation (males ≈1.9 years; females ≈2.4 years) of Lake Erie yellow perch populations (Purchase et al. 2005), and the exclusionary mechanism of the nets used (no fish < 15.24 cm) ensure that only adult yellow perch, well above the threshold for adult-related estrogen-sensitive SSD (Malison et al. 1985; Purchase et al. 2005), were sampled. Therefore, these results provide considerable insight into the seasonal and sex-specific endocrine status of adult yellow perch with implications for understanding SSD.

Female yellow perch sampled from Lake Erie for this study were on average heavier than males (Fig. 1) which is consistent with female-biased SSD in yellow perch (Best 1981). Females showed a clear positive relationship between body mass and age, with older yellow perch being heavier and younger yellow perch being lighter while males did not show a significant relationship between body mass and age. Correlation analyses (Table 2) suggest that small yellow perch would have higher liver *cyp19a1a* and IGF-Ib mRNA levels than large yellow perch, which could be indicative of a faster growth rate in smaller yellow perch. GH mRNA levels followed a pattern of higher levels in females and higher levels in spring (Fig. 2). Several studies have shown season or sex effects on GH expression in teleost species, such as salmonids (Björnsson 1997), gilthead sea bream (*Sparus aurata* L.; Meiri et al. 2004) and common carp (*Cyprinus carpio* L.; Figueroa et al. 2005). In regards to yellow perch, Roberts et al. (2004) reported that GH protein levels in pituitaries were significantly higher in May than in other months, with a tenfold increase compared to October. In further support of our findings with seasonal GH mRNA levels in Lake Erie yellow perch, Swift and Pickford (1965) found that pituitary homogenates collected from European perch (*Perca fluviatilis* L.) during April–June had higher growth-promoting ability than July–August in a bioassay system using fathead minnow (*Pimephales promelas* Rafinesque).

Although many studies have shown that GH stimulates the transcription and release of IGF-I from the liver (Biga et al. 2005; Carnevali et al. 2005), there is not a significant correlation between GH and IGF-Ib mRNA levels in this study (Table 2). In separate studies, both yellow perch (Jentoft et al. 2005) and the closely related Eurasian perch (Jentoft et al. 2004) showed a lack of responsiveness in hepatic IGF-I mRNA levels to exogenous GH treatment. This unresponsiveness to growth hormone treatment is not specific to perch, as barramundi (*Lates calcarifer* Bloch) also showed no response in IGF-I mRNA levels to GH treatment (Ståhlbom et al. 1999). Similarly, of the four alternative IGF-I transcripts identified in coho salmon (*Oncorhynchus kisutch* Walbaum) liver (Duguay et al. 1994), the ones which correspond to the two IGF-I transcripts in yellow perch (Lynn and Shepherd 2007) and Eurasian perch (Jentoft et al. 2004) are unresponsive to GH treatment. Further, while exogenous growth hormone did not stimulate growth in yellow perch, dietary E_2 treatment did stimulate growth (Jentoft et al. 2005), suggesting that the growth-promoting actions of E_2 may work independently of GH and/or IGF-I.

There was a significant effect of season on SL mRNA levels in yellow perch (Fig. 3), with higher levels occurring in spring rather than autumn, but studies on seasonal SL expression have produced rather conflicting results. In channel catfish (*Ictalurus punctatus* Rafinesque), SL mRNA levels in the largest adult class sampled were higher in April than in December (Tang et al. 2001). However, in rainbow trout (*Oncorhynchus mykiss* Walbaum), the highest levels of plasma SL in both mature male and mature female fish were in late summer (August) with a secondary peak in spring (April; Rand-Weaver et al. 1995). In masu salmon (*O. masou* Brevoort), during the second year of development, the highest SL mRNA levels were in late summer (August; Bhandari et al. 2003) but in gilthead sea bream the highest levels of plasma SL were very clearly in the winter (December; Mingarro et al. 2002). Perhaps seasonal SL expression levels are species dependent and related to the reproductive strategy of the fish, as there are studies that have shown SL to be modulated by GnRH and steroids (Rand-Weaver et al. 1992; Mayer et al. 1998).

There were significant differences between male and female I_H with females having a higher average I_H (>0.1) than males (≤0.1; Fig. 4), yet there was no effect of season or age on I_H. The year effect on sex-specific autumn I_H indicates that, while the sampling took place on the same day of the year, most probably, some environmental aspect was significantly different between the years (e.g., temperature, light, water quality, etc.). Both sex and season had a significant effect on liver IGF-Ib mRNA levels, with females in the autumn having lower levels than the other sex-specific seasonal groups (Fig. 5). Unfortunately, there are few studies that have examined seasonal expression of liver IGF-I and within those studies there are somewhat conflicting results, making interpretation difficult (Duan et al. 1995; Beckman et al. 1998).

Some of the most interesting results of this study involve liver ERs and liver *cyp19a1a* mRNA levels. Surprisingly, little work has been done on seasonal expression levels of liver ERs in fish despite their obvious role in reproduction and only recently have studies begun to examine the sex-specific expression levels of the various ERs in liver. Both liver *esr1* and liver *esr2a* mRNA levels were significantly influenced by sex in addition to a sex × season interaction; however, only liver *esr1* mRNA levels were significantly altered by sampling season (Figs. 6 and 7). Females clearly had higher mean liver *esr1* mRNA levels than males and males had higher mean liver *esr2a* mRNA levels than females. Halm et al. (2004) found very similar results to this study, with female European sea bass (*Dicentrarchus labrax* L.*)* having much higher liver ERα mRNA levels than males and males having higher liver ERβ1 mRNA levels than females. Only Sabo-Attwood et al. (2004) measured seasonal expression levels over a single year of all three ER mRNAs in female largemouth bass (*Micropterus salmoides* Lacepède) livers from October to March. In their study, liver ERα mRNA levels in females peaked in late winter (February–March) and liver ERγ (*esr2a*) mRNA levels changed very little over the 6-month sampling period with a slight increase during February to March. In yellow perch from this study, liver *esr1* mRNA levels had the strongest (positive) relationships with I_G (r=0.79) and ovarian *cyp19a1a* mRNA levels (r=0.72; Table 2). The livers of female spotted sea trout (*Cynoscion nebulosus* Cuvier) showed increased levels of ER mRNA (presumably ERα) during the summer months along with a corresponding increase in I_G (Smith and Thomas 1991). The relationship between liver *esr1* mRNA level and circulating estrogen is supported in the literature as the liver *esr1* gene is known to have an estrogen response element and to be upregulated in response to increased E_2 levels (Pakdel et al. 1997; Bowman et al. 2002), but such a relationship is as yet unknown in adult yellow perch. However, we have observed that dietary administration of E_2 in juvenile yellow perch results in elevated liver *esr1* mRNA levels (Lynn, unpublished data).

Both sex and season had significant effects on liver *cyp19a1a* mRNA levels in yellow perch (Fig. 8), with males being higher than females and levels being higher in spring than in autumn. There was also a significant effect of age, with older fish having lower liver *cyp19a1a* mRNA levels than younger fish within the same sex-specific seasonal group. The relationship between liver *cyp19a1a* and ovary *cyp19a1a* mRNA levels was significant with a negative correlation (Table 2), suggesting that liver *cyp19a1a* mRNA levels are tied to circulating estrogen levels and liver *cyp19a1a* could function as an intermediate between the ovarian estrogen and liver growth axes.

I_G (Fig. 9) and ovarian *esr1* (Fig. 10), but not *esr2a*, mRNA levels showed significant differences between seasons with larger ovaries in the autumn having lower ovarian *esr1* mRNA levels. Yellow perch ovarian *cyp19a1a* mRNA levels were significantly influenced by season with autumn levels being significantly higher than spring levels (Fig. 11). Several studies have linked follicular development or spawning and ovarian *cyp19a1a* mRNA levels or estrogen levels. A study in adult killifish (*Fundulus heteroclitus* L.) found that reproductively active females (May–July) had higher ovarian CYP19A1 (*cyp19a1a*) mRNA levels than reproductively inactive females (August–September; Greytak et al. 2005). Another study on fathead minnow found similar results with higher ovarian CYP19A1 mRNA levels in reproductively active females than non-reproductively active females (Villeneuve et al. 2006). Choi et al. (2005) found steadily increasing levels of CYP19A1 mRNA in ovaries of wrasse (*Halichoeres tenuispinis* Günther) from May to August with levels peaking during the spawning period in July and August. In addition, ovarian follicles in channel catfish showed higher levels of CYP19A1 mRNA corresponding with vitellogenesis during the winter months (February; Kumar et al. 2000; Yoon et al. 2008). In other work conducted in yellow perch, it was reported that monthly plasma E_2 concentrations in females from October to April were higher in autumn than spring, whereas testosterone levels showed an inverse pattern (low in autumn, high in spring; Dabrowski et al. 1996; Ciereszko et al. 1997). Also, yellow perch ovarian follicles showed significantly higher production of estradiol in October than any other month in the experimental period (October to April; Dabrowski et al. 2002). These results corroborate the findings of this study which showed significantly higher ovarian *cyp19a1a* mRNA levels in autumn (October) as compared to spring (May).

In this study, female yellow perch ovary *cyp19a1a* mRNA levels were negatively correlated with both pituitary GH and liver IGF-Ib mRNA levels (Table 2). Ovarian *cyp19a1a* mRNA levels at least partially control circulating E_2 levels as the ovary is the primary site of global E_2 synthesis. The implication is that, when E_2 levels in females are elevated in autumn (Dabrowski et al. 1996; Ciereszko et al. 1997), along with liver *esr1* mRNA levels, GH and liver IGF-Ib mRNA levels are depressed. Furthermore, liver *cyp19a1a* mRNA levels showed a significant negative correlation with I_G and liver *esr1* and ovary *cyp19a1a* mRNA levels, indicating that when ovaries are largest (in autumn) *cyp19a1a* mRNA levels are higher, leading to greater E_2 production, as supported by the studies of Ciereszko et al. (1997) and Dabrowski et al. (1996). This could likely result in the lower liver *cyp19a1a* mRNA levels and higher liver *esr1* mRNA levels seen in this study, ultimately producing lower mRNA levels of growth-regulating hormones (i.e., GH and IGF-Ib). While the correlations are significant, the true nature of the relationships

between these parameters requires further investigation. Moreover, although this pattern of gene expression does not support estrogen-stimulated SSD in yellow perch, it is important to remember that these are baseline endogenous levels of gene expression (not in response to exogenous administration of E_2) and not measurements of the protein or steroid levels.

In summary, we report significant sex-, tissue-, and seasonal-specific changes in the mRNA levels of most of the key endocrine genes measured (GH, PRL, SL, IGF-Ib, liver *esr1*, liver *esr2a*, liver *cyp19a1a*, ovary *esr1*, ovary *esr2a*, and ovary *cyp19a1a*) in adult yellow perch from Lake Erie. These results indicate that both male and female yellow perch have increased mRNA levels of growth-regulating hormones (GH and IGF-Ib) in spring as opposed to autumn, indicating the importance of spring in yearly growth. Also, there is a distinct difference in male and female liver ER (*esr1* and *esr2a*) mRNA levels and liver *esr1* was significantly influenced by season. Lastly, ovarian *cyp19a1a* mRNA levels (a possible indicator of plasma E_2 levels) showed a significant negative correlation with GH, IGF-Ib, and liver *cyp19a1a* mRNA levels and a significant positive correlation with liver *esr1* mRNA levels and I_G in females. These results indicate new avenues for research related to seasonal and sex-specific expression of these genes in fish and also provide further insight into potential endocrine pathways involved with yellow perch SSD.

Acknowledgments Special thanks go to J. Swartz of Swartz Fisheries and his crew for being so helpful in the attainment of samples. D. Klarer and the staff at Old Woman Creek NERR/SNP, Huron OH, USA, provided lodging and laboratory facilities. B. O'Hara, University of Kentucky, helped in the implementation of the qPCR assays. This work was funded by NOAA National Estuarine Research Reserve Graduate Student Fellowship #NA16OR2400 to S. G. Lynn. This study was also funded, in part, by grants from the National Research Initiative Competitive Grants Program/USDA award no. 2002-35206-11629 and 2004-05124 and the support of the US Geological Survey and Kentucky Water Resources Research Institute Grant Agreement No. 01HQGR0133 to B.S. Shepherd and ARS/USDA CRIS #3655-31000-020-00D. The views contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the US Government. Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable. This manuscript is submitted for publication with the understanding that the US Government is authorized to reproduce and distribute reprints for governmental purposes.

References

Arukwe A (2006) Toxicological housekeeping genes: do they really keep the house? Environ Sci Technol 40:7944–7949

Beckman B, Larsen D, Moriyama S, Lee-Pawlak B, Dickhoff W (1998) Insulin-like growth factor-I and environmental modulation of growth during smoltification of spring Chinook salmon (*Oncorhynchus tshawytscha*). Gen Comp Endocrinol 109:325–335

Best C (1981) Initiation of artificial feeding and the control of sex differentiation in yellow perch, *Perca flavescens*. Oceanography and Limnology, M.S. Thesis. University of Wisconsin-Madison, Madison, WI. p. 121

Bhandari R, Taniyama S, Kitahashi T, Ando H, Yamauchi K, Zohar Y, Ueda H, Urano A (2003) Seasonal changes of responses to gonadotropin-releasing hormone analog in expression of growth hormone/prolactin/somatolactin genes in the pituitary of masu salmon. Gen Comp Endocrinol 130:55–63

Biga PR, Peterson BC, Schelling GT, Hardy RW, Cain KD, Overturf K, Ott TL (2005) Bovine growth hormone treatment increased IGF-I in circulation and induced the production of a specific immune response in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 246:437–445

Björnsson B (1997) The biology of salmon growth hormone: from daylight to dominance. Fish Physiol Biochem 17:9–24

Bowman C, Kroll K, Gross T, Denslow N (2002) Estradiol-induced gene expression in largemouth bass (*Micropterus salmoides*). Mol Cell Endocrinol 196:67–77

Bustin SA (2000) Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endocrinol 25:169–193

Carnevali O, Cardinali M, Maradonna F, Parisi M, Olivotto I, Polzonetti-Magni AM, Mosconi G, Funkenstein B (2005) Hormonal regulation of hepatic IGF-I and IGF-II gene expression in the marine teleost *Sparus aurata*. Mol Reprod Dev 71:12–18

Chen S-L, Li J, Deng S-P, Tian Y-S, Wong O-Y, Zhuang Z-M, Sha Z-X, Xu J-Y (2007) Isolation of female-specific AFLP markers and molecular identification of genetic sex in half-smooth tongue sole (*Cynoglossus semilaevis*). Mar Biotechnol 9:273–280

Choi C, Habibi H (2003) Molecular cloning of estrogen receptor a and expression pattern of estrogen receptor subtypes in male and female goldfish. Mol Cell Endocrinol 204:169–177

Choi J, Park J, Jeong H, Lee Y, Takemura A, Kim S (2005) Molecular cloning of cytochrome P450 aromatases in the protogynous wrasse, *Halichoeres tenuispinis*. Comp Biochem Physiol B Bio Mol Biol 141:49–59

Ciereszko RE, Dabrowski K, Ciereszko A, Ebeling J, Ottobre JS (1997) Effects of temperature and photoperiod on reproduction of female yellow perch *Perca flavescens*: plasma concentrations of steroid hormones, spontaneous and induced ovulation and quality of eggs. J World Aquac Soc 28:344–356

Dabrowski K, Ciereszko RE, Ciereszko A, Toth GP, Christ SA, El-Saidy D, Ottobre JS (1996) Reproductive physiology of yellow perch (*Perca flavescens*): environmental and endocrinological cues. J Appl Ichthyol 12:139–148

Dabrowski K, Ciereszko RE, Ciereszko A, Ottobre JS (2002) In vitro production of ovarian steroids in yellow perch (*Perca flavescens*): effects of photothermal manipulation, gonadotropin and phorbol ester. Reprod Biol 2:163–186

Devlin RH, Nagahama Y (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208:191–364

DeVries DR, Frie RV (1996) Determination of age and growth. In: Murphy BR, Willis DW (eds) Fisheries techniques. American Fisheries Society, Bethesda, pp 463–512

Dheda K, Huggett JF, Chang JS, Kim LU, Bustin SA, Johnson MA, Rook GAW, Zumla A (2005) The implications of using an inappropriate reference gene for real-time reverse transcription PCR data normalization. Anal Biochem 344:141–143

Duan C (1997) The insulin-like growth factor system and its biological actions in fish. Am Zool 37:491–503

Duan C, Plisetskaya EM, Dickhoff WW (1995) Expression of insulin-like growth factor I in normally and abnormally

developing coho salmon (*Oncorhynchus kisutch*). Endocrinology 136:446–452

Duguay SJ, Swanson P, Dickhoff WW (1994) Differential expression and hormonal regulation of alternatively spliced IGF-I mRNA transcripts in salmon. J Mol Endocrinol 12:25–37

Figueroa J, San Martín R, Flores C, Grothusen H, Kausel G (2005) Seasonal modulation of growth hormone mRNA and protein levels in carp pituitary: evidence for two expressed genes. J Comp Physiol B 175:185–192

Freeman ME, Kanyicska B, Lerant A, Nagy G (2000) Prolactin: structure, function, and regulation of secretion. Physiol Rev 80:1523–1631

Fukada H, Ozaki Y, Pierce AL, Adachi S, Yamauchi K, Hara A, Swanson P, Dickhoff WW (2005) Identification of the salmon somatolactin receptor, a new member of the cytokine receptor family. Endocrinology 146:2354–2361

González A, Piferrer F (2003) Aromatase activity in the European sea bass (*Dicentrarchus labrax* L.) brain. Distribution and changes in relation to age, sex, and the annual reproductive cycle. Gen Comp Endocrinol 132:223–230

Greytak S, Champlin D, Callard G (2005) Isolation and characterization of two cytochrome P450 aromatase forms in killifish (*Fundulus heteroclitus*): differential expression in fish from polluted and unpolluted environments. Aquat Toxicol 71:371–389

Guiguen Y, Baroiller J, Ricordel M, Iseki K, McMeel O, Martin S, Fostier A (1999) Involvement of estrogens in the process of sex differentiation in two fish species: the rainbow trout (*Oncorhynchus mykiss*) and a tilapia (*Oreochromis niloticus*). Mol Reprod Dev 54:154–162

Haidle L, Janssen JE, Gharbi K, Moghadam HK, Ferguson MM, Danzmann RG (2008) Determination of quantitative trait loci (QTL) for early maturation in rainbow trout (*Oncorhynchus mykiss*). Mar Biotechnol. doi:10.1007/s10126-008-9098-5

Halm S, Martínez-Rodríguez G, Rodríguez L, Prat F, Mylonas C, Carrillo M, Zanuy S (2004) Cloning, characterisation and expression of three oestrogen receptors (ERa, ERb1 and ERb2) in the European sea bass, *Dicentrarchus labrax*. Mol Cell Endocrinol 223:63–75

Hayward RS, Wang N (2001) Failure to induce over-compensation of growth in maturing yellow perch. J Fish Biol 59:126–140

Hile R, Jobes FW (1942) Age and growth of the yellow perch, *Perca flavescens* (Mitchell), in Wisconsin waters of Green Bay and northern Lake Michigan. Pap Mich Acad Sci Arts Lett 27:241–266

Jentoft S, Aastveit AH, Andersen Ø (2004) Molecular cloning and expression of insulin-like growth factor-I (IGF-I) in Eurasian perch (*Perca fluviatilis*): lack of responsiveness to growth hormone treatment. Fish Physiol Biochem 30:67–76

Jentoft S, Topp N, Seeliger M, Malison JA, Barry TB, Held JA, Roberts S, Goetz F (2005) Lack of growth enhancement by exogenous growth hormone treatment in yellow perch (*Perca flavescens*) in four separate experiments. Aquaculture 250:471–479

Kinnunen RE (2003) Great lakes commercial fisheries. Michigan Sea Grant Extension, Marquette, p 19

Kumar R, Ijiri S, Trant J (2000) Changes in the expression of genes encoding steroidogenic enzymes in the channel catfish (*Ictalurus punctatus*) ovary throughout a reproductive cycle. Biol Reprod 63:1676–1682

Lynn SG, Shepherd BS (2007) Molecular characterization and sex-specific tissue expression of prolactin, somatolactin and insulin-like growth factor-I in yellow perch (*Perca flavescens*). Comp Biochem Physiol B Biochem Mol Biol 147:412–427

Lynn SG, Birge WJ, Shepherd BS (2008) Molecular characterization and sex-specific tissue expression of estrogen receptor α (esr1), estrogen receptor β a (esr2a), and ovarian aromatase (cyp19a1a) in yellow perch (*Perca flavescens*). Comp Biochem Physiol B Biochem Mol Biol 149:126–147

Malison JA (1999) A white paper on the status and needs of yellow perch aquaculture in the North Central Region. North Central Regional Aquaculture Center, Madison, p 20

Malison JA, Best CD, Kayes TB, Amundson CH (1985) Hormonal growth promotion and evidence for size-related differences 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 (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 (1988) Growth and feeding responses of male versus female yellow perch (*Perca flavescens*) treated with estradiol 17β. Can J Fish Aquat Sci 45:1942–1948

Mayer I, Rand-Weaver M, Borg B (1998) Effects of gonadectomy and steroids on plasma and pituitary levels of somatolactin in Atlantic salmon, *Salmo salar*. Gen Comp Endocrinol 109: 223–231

Meiri I, Knibb W, Zohar Y, Elizur A (2004) Temporal profile of β follicle-stimulating hormone, β luteinizing hormone, and growth hormone gene expression in the protandrous hermaphrodite, gilthead sea bream, *Sparus aurata*. Gen Comp Endocrinol 137:288–299

Melo A, Ramsdell J (2001) Sexual dimorphism of brain aromatase activity in medaka: induction of a female phenotype by estradiol. Environ Health Perspect 109:257–264

Menuet A, Anglade I, Le Guevel R, Pellegrini E, Pakdel F, Kah O (2003) Distribution of aromatase mRNA and protein in the brain and pituitary of female rainbow trout: comparison with estrogen receptor alpha. J Comp Neurol 462:180–193

Mingarro M, Vega-Rubín de Celis S, Astola A, Pendón C, Valdivia M, Pérez-Sánchez J (2002) Endocrine mediators of growth and feeding behaviour in gilthead sea bream (*Sparus aurata*): growth hormone and somatolactin paradigm. Gen Comp Endocrinol 128:102–111

Nicholas KB, Nicholas HB, Deerfield DW II (1997) GeneDoc: analysis and visualization of genetic variation. embnet.news 4:14

Pakdel F, Delaunay F, Ducouret B, Flouriot G, Kern L, Lazennec G, Le Dréan Y, Petit F, Salbert G, Saligaut D, Tujague M, Valotaire Y (1997) Regulation of gene expression and biological activity of rainbow trout estrogen receptor. Fish Physiol Biochem 17:123–133

Patil JG, Hinze SJ (2008) Simplex PCR assay for positive identification of genetic sex in the Japanese medaka, *Oryzias latipes*. Mar Biotechnol. doi:10.1007/s10126-008-9106-9

Purchase CF, Collins NC, Morgan GE, Shuter BJ (2005) Sex-specific covariation among life-history traits of yellow perch (*Perca flavescens*). Evol Ecol Res 7:549–566

Rand-Weaver M, Swanson P, Kawauchi H, Dickhoff WW (1992) Somatolactin, a novel pituitary protein: purification and plasma levels during reproductive maturation of coho salmon. J Endocrinol 133:393–403

Rand-Weaver M, Pottinger T, Sumpter J (1995) Pronounced seasonal rhythms in plasma somatolactin levels in rainbow trout. J Endocrinol 146:113–119

Roberts S, Barry T, Malison J, Goetz F (2004) Production of a recombinantly derived growth hormone antibody and the characterization of growth hormone levels in yellow perch. Aquaculture 232:591–602

Sabo-Attwood T, Kroll K, Denslow N (2004) Differential expression of largemouth bass (*Micropterus salmoides*) estrogen receptor isotypes alpha, beta, and gamma by estradiol. Mol Cell Endocrinol 218:107–118

Schott EF, Kayes TB, Calbert HE (1978) Comparative growth of male versus female yellow perch fingerlings under controlled environmental conditions. Am Fish Soc Spec Publ 11:181–186

Sellars MJ, Vuocolo T, Leeton LA, Coman GJ, Degnan BM, Preston NP (2007) Real-time RT-PCR quantification of Kuruma shrimp transcripts: a comparison of relative and absolute quantification procedures. J Biotechnol 129:391–399

Shepherd BS, Sakamoto T, Nishioka RS, Richman NH, Mori I, Madsen SS, Chen TT, Hirano T, Bern HA, Grau EG (1997) Somatotropic actions of the homologous growth hormone and prolactins in the euryhaline teleost, the tilapia, *Oreochromis mossambicus*. Proc Natl Acad Sci U S A 94:2068–2072

Smith J, Thomas P (1991) Changes in hepatic estrogen-receptor concentrations during the annual reproductive and ovarian cycles of a marine teleost, the spotted sea trout, *Cynoscion nebulosus*. Gen Comp Endocrinol 81:234–245

Sokal RR, Rohlf FJ (1995) Biometry, the principles and practice of statistics in biological research, 3rd edn. Freeman, San Francisco, p 887

Ståhlbom AK, Sara VR, Hoeben P (1999) Insulin-like growth factor mRNA in barramundi (*Lates calcarifer*): alternative splicing and nonresponsiveness to growth hormone. Biochem Genet 37:69–93

Swift DR, Pickford GE (1965) Seasonal variations in the hormone content of the pituitary gland of the perch, *Perca fluviatilis* L. Gen Comp Endocrinol 5:354–365

Tang Y, Shepherd B, Nichols A, Dunham R, Chen T (2001) Influence of environmental salinity on mRNA levels of growth hormone, prolactin and somatolactin in the pituitary of the channel catfish, *Ictalurus punctatus*. Mar Biotechnol 3:205–217

Troutman MB (1980) The fishes of Ohio. Ohio State University Press and the Ohio Sea Grant Program and Center for Lake Erie Area Research, Columbus, p 683

Villeneuve D, Knoebl I, Kahl M, Jensen K, Hammermeister D, Greene K, Blake L, Ankley G (2006) Relationship between brain and ovary aromatase activity and isoform-specific aromatase mRNA expression in the fathead minnow (*Pimephales promelas*). Aquat Toxicol 76:353–368

Vong QP, Chan KM, Cheng CHK (2003) Quantification of common carp (*Cyprinus carpio*) IGF-I and IGF-II mRNA by real-time PCR: differential regulation of expression by GH. J Endocrinol 178:513–521

Yoon SH, Itoh Y, Kaneko G, Nakaniwa M, Ohta M, Watabe S (2008) Molecular characterization of Japanese sillago vitellogenin and changes in its expression levels on exposure to 17β-estradiol and 4-tert-octylphenol. Mar Biotechnol (NY) 10:19–30

Zar JH (1999) Biostatistical analysis. Prentice-Hall, Upper Saddle River, p 931