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ARTICLE

Identification of Gender in Yellow Perch by External Morphology: Validation in Four Geographic Strains and Effects of Estradiol

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Abstract
External morphological criteria that enable the rapid determination of gender have been developed for Yellow Perch *Perca flavescens*. Criteria are based upon (1) shape of the urogenital papilla (UGP), (2) size of the UGP relative to the anal (AN) opening, and (3) coloration of the UGP. In females, the UGP appeared (1) rounded at the anterior margin, (2) pointed at the posterior margin and had a V or U shape (crescent shaped in mature animals), (3) generally lacked reddish coloration, and (4) narrower relative to the AN. In males, the UGP (1) appeared circular or oval around the entire margin, (2) typically displayed reddish coloration, and (3) was generally wider relative to the AN. To verify accuracy of these criteria, gender was verified internally in perch of various sizes, sex, and maturity from four domesticated geographic strains (n = 1,389). For all perch tested, accuracy was 97.3% for both sexes, 98.8% for females, and 95.9% for males. To experimentally verify accuracy of these criteria, juvenile Yellow Perch (n = 913) were treated with dietary 17β-estradiol (E2; 15 mg/kg diet) or a control diet. Accuracy was 97.7% for control females and 95.1% for control males, which diminished to 63.9% for the E2-treated females and 57.6% for E2-treated males. We developed a gender identification algorithm that will enable sorting of sexes for many uses including (1) collection of broodstock by new aquaculture producers, (2) management of broodstocks for existing producers, (3) improved selection criteria for genetic selection programs, (4) studies on gender-specific differences in Yellow Perch physiology, and (5) the unharmed release of fish in field and aquaculture settings. Uncoupling of external UGP morphology from actual gender in E2-treated perch can enable producers and biologists to detect exposure to estrogenic compounds in areas where endocrine disruption is suspected.

The ability to determine gender in finfish has been essential to the successful development of fisheries management, basic research, and aquaculture. However, reliable external identification methods have not been developed for many important finfish species. While gender can be identified by the release of sperm or eggs from sexually mature fish, such criteria cannot be used outside of the spawning season and is not applicable for juveniles, sexually immature adults, or sexually monomorphic species. Therefore, the need for reliable determination of gender and reproductive stage has driven the development of diagnostic

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methods involving the use of catheterization or biopsies (Ross 1984), biochemical methods (Le Bail and Breton 1981; Craik and Harvey 1984; Pottinger et al. 2005), molecular markers (Devlin et al. 1994; Griffiths et al. 2000; Nagler et al. 2004), and imaging techniques (e.g., ultrasonography, endoscopy, and laparoscopy; Moccia et al. 1984; Ortenburger et al. 1996; Novelo and Tiersch 2012) for a number of finfish species. While such methods are generally precise, there are drawbacks such as the need for laboratory infrastructure that is not available to all researchers or aquaculture producers and deployment of complicated equipment and procedures in a field or aquaculture setting. Such methods are time consuming, require substantial expertise, and can involve stress and injury to the fish. Consequently, methods that enable rapid, accurate identification of gender in juvenile and adult finfish, without equipment or complicated procedures, are highly desirable.

The development of criteria for external gender identification using external morphology (e.g., body shape, genital papilla), body coloration, or both has only been described for very few stenohaline freshwater species in North America. These would include Fathead Minnow *Pimephales promelas* (Ankley et al. 2001), Bluegill *Lepomis macrochirus* (McComish 1968), Largemouth Bass *Micropterus salmoides* (Parker 1971), Northern Pike *Esox lucius* (Casselman 1974), and Muskellunge *E. masquinongy* (Lebeau and Pageau 1989). While the methods described for Northern Pike and Muskellunge appear to be the only ones that are sufficiently robust to enable gender identification in immature fish, comparatively less is known for percid fishes.

Yellow Perch *Perca flavescens* are an ecologically and economically important percid with a wide geographic distribution in North America. One particular aspect of Yellow Perch physiology is that females grow larger and faster than males and this growth can be estrogen-dependent (Malison et al. 1985, 1988; Goetz et al. 2009). Given there are differences in growth rate and in the functional relationships between body weight and length (and maturation) among geographic strains of Yellow Perch (Brown et al. 2002; Wang et al. 2009; Rosauer et al. 2011), one could hypothesize that differences occur in the external morphology of the urogenital papilla (UGP) between strains, juveniles, and adults.

Based on this knowledge, we developed criteria for the external identification of gender in Yellow Perch using fish from four geographic strains (Choptank River, Maryland; Perquimans River, North Carolina; Sassafras River, Maryland; Lake Winnebago, Wisconsin) that vary in size and maturity. We also experimentally tested the validity of these criteria using estrogen-treated juvenile perch (Malison et al. 1985; Goetz et al. 2009). The present study describes external morphology of the UGP of male and female Yellow Perch, criteria for determining gender using UGP morphology, a decision algorithm to enable systematic identification of gender in these geographic perch strains, and observational and experimental data on the accuracy of these criteria.

METHODS

General conditions for animal husbandry.—Yellow Perch were maintained and euthanized in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Wisconsin– Milwaukee. All perch were obtained from captive broodstocks maintained at the School of Freshwater Sciences (Milwaukee, Wisconsin). These broodstocks were originally derived from gametes taken from wild Yellow Perch from several geographic locations, including the Choptank River (Chesapeake Bay, Maryland), Perquimans River (Albemarle Sound, North Carolina), Sassafras River (Chesapeake Bay, Maryland), and Lake Winnebago (Fond du Lac, Wisconsin) (Grzybowski et al. 2010; Rosauer et al. 2011). Spawning of adults, production of feed-trained fingerlings, and general husbandry for the Choptank, Perquimans, and Winnebago strains have been previously described (Rosauer et al. 2011). Gonadal somatic index (GSI) in many of the perch used for this work was generally minimal (<5%) as several groups (with exception as described below) had not undergone the environmental cycling required for sexual maturation (Hokanson 1977). Gonadal somatic index was determined from random samples for specific strains only (as reported below or in the Results section). The GSI was calculated as follows (Morrison et al. 1985):

$$\text{GSI} = \text{gonad weight in grams} \times (\text{body weight in grams} - \text{gonad weight in grams})^{-1} \times 100.$$

Specific rearing conditions for Choptank, Perquimans, Winnebago, and Sassafras strains.—Yellow Perch from the Perquimans and Choptank strains were F_3 progeny derived from parents (F_2 broodstock) spawned in March and April 2011, respectively. Data on sex identification for the Choptank and Perquimans perch were obtained from an on-going growth performance trial that is part of a broodstock genetic improvement program. Conditions for the performance trial were similar to those previously described (Rosauer et al. 2011), but perch (~48 per strain) were sampled at 6 and 9 months of age and maintained on a high protein–high energy diet (pellet size, 3.0-mm; Bio Vita Fry, Bio-Oregon, Longview, Washington); GSI was not determined for these perch.

To experimentally validate UGP morphological criteria, additional F_3 progeny were treated with dietary 17β-estradiol (E2). Approximately 70 Yellow Perch from the Choptank (3.0 ± 0.2 g, mean ± standard error of the mean) strain and 70 perch from the Perquimans (6.4 ± 0.2 g) strain were separately stocked into eight, circular, 120-L fiberglass tanks per strain ($n = 913$ total fish). Tanks were blocked by strain (Choptank or Perquimans) and treatment (control and estrogen), resulting in four replicate tanks consisting of Choptank control, Choptank E2, Perquimans control, and Perquimans E2. Tanks were supplied with flow-through water at 20 ± 1.5°C and photoperiod was maintained at 16 h light : 8 h dark. Yellow Perch

were acclimated for a period of 3 weeks. Following acclimation, the perch were weighed and measured and fed a diet containing 15 mg E2 (Sigma-Aldrich, St. Louis, Missouri) per kilogram of diet (pellet size, 2.0 mm; Bio Vita Fry, Bio-Oregon,) or a control diet treated with vehicle (ethanol) alone. Diets were prepared as previously described (Goetz et al. 2009) and perch were fed by hand to apparent satiation twice daily. At the end of the 15-week period, all perch in each tank were anesthetized with tricaine methanesulfonate (MS-222; 100 mg/L), weighed and measured, and euthanized by decapitation. Gender was determined externally for all perch at this time (criteria described below) followed by internal confirmation of gender by examination of the gonad (fish were 11 months old at this time). Gonads were removed from random samples and preserved in zinc-buffered formalin (10%) fixative (Z-fix, Anatech, Battle Creek, Michigan) for subsequent histological analyses. To determine GSI, fixed gonads were blotted dry on paper towel and then weighed to the nearest 0.001 g using a calibrated analytical balance (Voyager Pro, Ohaus, Parsippany, New Jersey). Gonad weights were corrected to account for weight loss (Anderson and Neumann 1996).

Two additional groups of F_3 Choptank Yellow Perch, not used for the growth performance trial, were also tested for gender identification. The first group represented perch that were held under similar conditions (water source, temperature, and photoperiod) as previously reported (Rosauer et al. 2011) and were 12 months old at the time of sampling. The second group of perch were separately maintained in a 2,000-L fiberglass tank and fed a maintenance ration (2% of biomass per day) using a commercially available feed (3.0 mm; BioVita Fry, Bio-Oregon). Photoperiod was maintained at 14 h light: 10 h dark and temperature was allowed to fluctuate over the seasons, but never exceeded 22°C in summer or went below 10°C in the winter. This second group was 13 months old at the time of sampling. At the time of sampling for sex identification, gonads were weighed from a subsample of these excess Choptank male and female perch for GSI determination; GSI was 1.57 ± 0.76% for females and 3.77 ± 0.82% for males.

Yellow Perch that comprise the Sassafras strain (24 months old) were obtained as feed-trained fingerlings in April 2010 and were held in a circular 2,400-L fiberglass tank. Photoperiod was maintained at 14 h light: 10 h dark and temperature was allowed to fluctuate over the seasons, but never exceeded 22°C in summer or went below 10°C in the winter. Once the perch reached a size of 40 g body weight, they were maintained on a maintenance ration of 2% biomass/d (3.0 mm; Bio Vita Fry). At the time of sampling, many of the male perch were spermiating. Consequently, a subsample of gonads from each sex was weighed to determine GSI; GSI was highly variable in females (8.6 ± 5.6%) compared with males (2.54 ± 0.2%).

Winnebago strain Yellow Perch were F_2 progeny resulting from parental crosses of genetically selected broodstock (F_1) spawned in May 2009. Perch were reared, maintained, fed, and environmentally cycled as previously described (Rosauer et al. 2011). Perch were sampled for gender identification at 39 months of age. While these perch were confirmed to have spawned in May 2011 and May 2012, individual spawning events were not recorded for May 2012. Because these perch had been cycled for spawning, gonads were randomly sampled for weight to determine GSI; female GSI was 4.0 ± 1.5% and male GSI was 0.2 ± 0.01%.

Morphological criteria for external gender identification.—The morphological criteria used to externally determine gender in Yellow Perch from these various geographic strains were (1) shape of the UGP, (2) coloration of the UGP, and (3) size of the UGP relative to that of the anus (AN). After determination of sex had been made, the perch were euthanized as previously described and confirmation of sex was accomplished by internal examination of the gonads according to initial criteria described by Parker (1942) and expanded criteria reported by Malison et al. (1986). Since the work of Parker (1942) demonstrated that genital and urinary systems share a single duct, we have called this area where the urogenital opening occurs, the UGP.

Image capture and processing.—Images of the UGP and gonads were taken using a Canon Power Shot SX40HS camera equipped with a 35 × , 24–840-mm zoom lens (f-stop, 2.7–5.8). Images were imported into Adobe Photoshop CS6 (version 13.0, 64 bit, San Jose, California) for processing, zooming, and cropping. To ensure the best possible representation of coloration and appearance of the AN and UGP regions, images were processed using the clone stamp tool to reduce reflected light (see Figure 1) and white- and color-balancing (see Figure 2) to compensate for variable lighting conditions (Adobe Photoshop CS6). Adobe Illustrator (16.0.0) was used for organization of the images.

Statistical analyses for datasets of untreated and estrogen-treated fish.—A completely randomized design (CRD) was used to examine and compare external and internal sex identification of four strains of untreated Yellow Perch for three size categories (see Table 1). The strains examined were Choptank, Perquimans, Sassafras, and Winnebago. The size categories (determined from overall size distributions) were defined in terms of TL and were: 80–170 mm, 171–210 mm, and 211–290 mm. Since perch were used from various populations, specific size categories are absent for certain geographic strains.

To answer questions regarding the efficacy of using external gender identification for untreated Yellow Perch, two analyses were performed. The first test was a binomial proportions test to determine whether external gender identification is effective in predicting the true gender of the perch, as determined by internal gender confirmation, over all strains, size categories, and true gender of the perch. The dependent, or Y-variable, was binary (1 = correct external gender identification and 0 = incorrect). The binomial proportions analysis compared the frequency count of the number of correctly identified external gender identifications to the number of incorrect external gender identifications.

The second test was a logistic analysis for determining whether strain, size category, or true gender of the perch influences the success of external gender identification. The same binary dependent variable was used as described above. The independent classification variables—Internal Gender

Confirmation, Strain, and Size Category—were used. If a significant ($P < 0.05$) Wald chi-square test was obtained, differences of least squares means on maximum likelihood estimates of the effects was used as a pairwise multiple comparison procedure. Odds ratios were obtained and used to determine the probability of correct external sex identification compared with the probability of incorrect identification for each comparison.

A CRD was used to examine and compare external and internal gender identification of two strains of estrogen-treated Yellow Perch for two treatments (see Table 2). The strains examined were Choptank and Perquimans, and the treatments applied were identified as Control and E2. As described above, a binomial proportions test was used to determine whether external gender identification is effective in predicting the true gender of the perch. Significant differences were declared at $P \leq 0.05$ from Wald chi-square tests. Odds ratios were obtained and used to determine the probability of correct external gender identification compared with the probability of incorrect identification for each significant effect comparison. A first logistic analysis used a three-factor full model with interactions of the categorical variables: Strain (Choptank and Perquimans), True Gender of the perch (based on internal examination, female or male), and Treatment (control or E2), to determine which factors influence the success of external gender identification in Yellow Perch. A second logistic analysis further examined odds ratios for significant main effects from the first analysis.

Analysis of the GSI of the Choptank and Perquimans Yellow Perch in the dietary E2 study were analyzed separately by two-way ANOVA using transformed data, with treatment (control or E2) and sex (female or male) as independent variables (main effects). Following significant ANOVA differences ($P < 0.05$), pairwise comparisons were performed using the Tukey–Kramer method.

In all cases, the stated P-value ($P < 0.05$) for individual comparisons is a general cut-off value (Steel and Torrie 1980). All values are reported as the mean, range, raw counts, or percentages. Analyses were performed using PC SAS software (version 9.2, 2002–2008; SAS Institute, Inc., Cary, North Carolina).

RESULTS

Appearance of the UGP in Untreated Female and Male Yellow Perch

The UGP is located just posterior to the AN opening (Figure 1). In females (Choptank, Perquimans, and Sassafras strains) of all size categories (80–170 mm, 171–210 mm, and 211–290 mm TL), the margin of UGP typically occurs in a "U" or "V" shape that is oriented towards the anal fin, and the width of the UGP is equivalent to, or narrower than, that of the AN opening (Figure 1B and 1D). Color of the UGP in these smaller Yellow Perch is either pale (Figure 1D) or light brown (Figure 1B). In the females sampled from the Winnebago strain (211–290 mm TL), the margin of the UGP had either a U shape or crescent shape (Figure 1F) and appeared dark brown, but was always equivalent (or less) in width to that of the AN margin. In addition to the crescent-shaped appearance of the UGP in the Winnebago strain, another unique observation for this strain was the presence of AN swelling in females (Figure 1F), which was not observed in similarly sized perch from the Choptank, Perquimans, or Sassafras strains (Figure 1A–D; in each panel, an inset figure shows the gonads of the corresponding perch).

In male Yellow Perch of all strains and sizes, the margin of the UGP was typically round or oval in shape, wider than that of the AN opening, and coloration ranged from pale brown to red (Figure 1A, C, E). Interestingly, we also observed AN swelling (Figure 1E) in males of the Winnebago strain (211–290 mm TL), which was not observed in similarly sized males from the Choptank, Perquimans or Sassafras strains.

Appearance of the UGP and Gonads of Yellow Perch Treated with E2

The UGP (and gonads) of control males and females (Figure 2A, B) of both strains (Choptank and Perquimans) appeared as described for untreated Yellow Perch shown in Figure 1A–D. By contrast, external features of the UGP in E2-treated males and females (Choptank and Perquimans strains) were generally similar in appearance (Figure 2C, D). Specifically, the UGP of E2-treated males (Figure 2C) had less developed coloration (similar to that of control females, Figure 1B), was U shaped and the width appeared equal to that of the AN. The margin of the UGP in E2-treated females appeared as a U or V shape, but the width of the UGP margin often appeared to be greater than that of the AN opening (Figure 2D). In each figure panel, an inset figure shows the gonads of the corresponding control and E2-treated males and females (Figure 1A–D). While confirming gender internally, gross examination of the gonads revealed that the testes and ovaries of E2-treated perch were smaller in size (Figure 2E, F). Specifically, the testes of E2-treated males (Figure 2C, E) were translucent, less developed, and difficult to detect. While histology was not performed on the gonads from these fish, the stage of development could be approximated macroscopically. The maturity stage of E2-treated male Yellow Perch most closely resembled the "resting stage" as described by Turner (1919) or "sterile" as described by Malison et al. (1986). The ovaries of E2-treated females (Figure 2D, F) were also less developed, but often displayed a hemorrhagic red coloration and the posterior was filled with a clear fluid. The maturity stages of control and E2-treated female perch are best described as being in stage III (developing early) and stage IID (maturing virgin), respectively (Treasurer and Holliday 1981).

Analyses of External Gender Identification Criteria for all Strains and Size Categories in Untreated Yellow Perch

Data on the accuracy of external gender identification in untreated male and female Yellow Perch were obtained from a total of 1,389 perch across all strains and sizes (Table 1). When evaluating data summarized in Table 1 (untreated, $n =$ 1,389), there was a highly significant difference between the

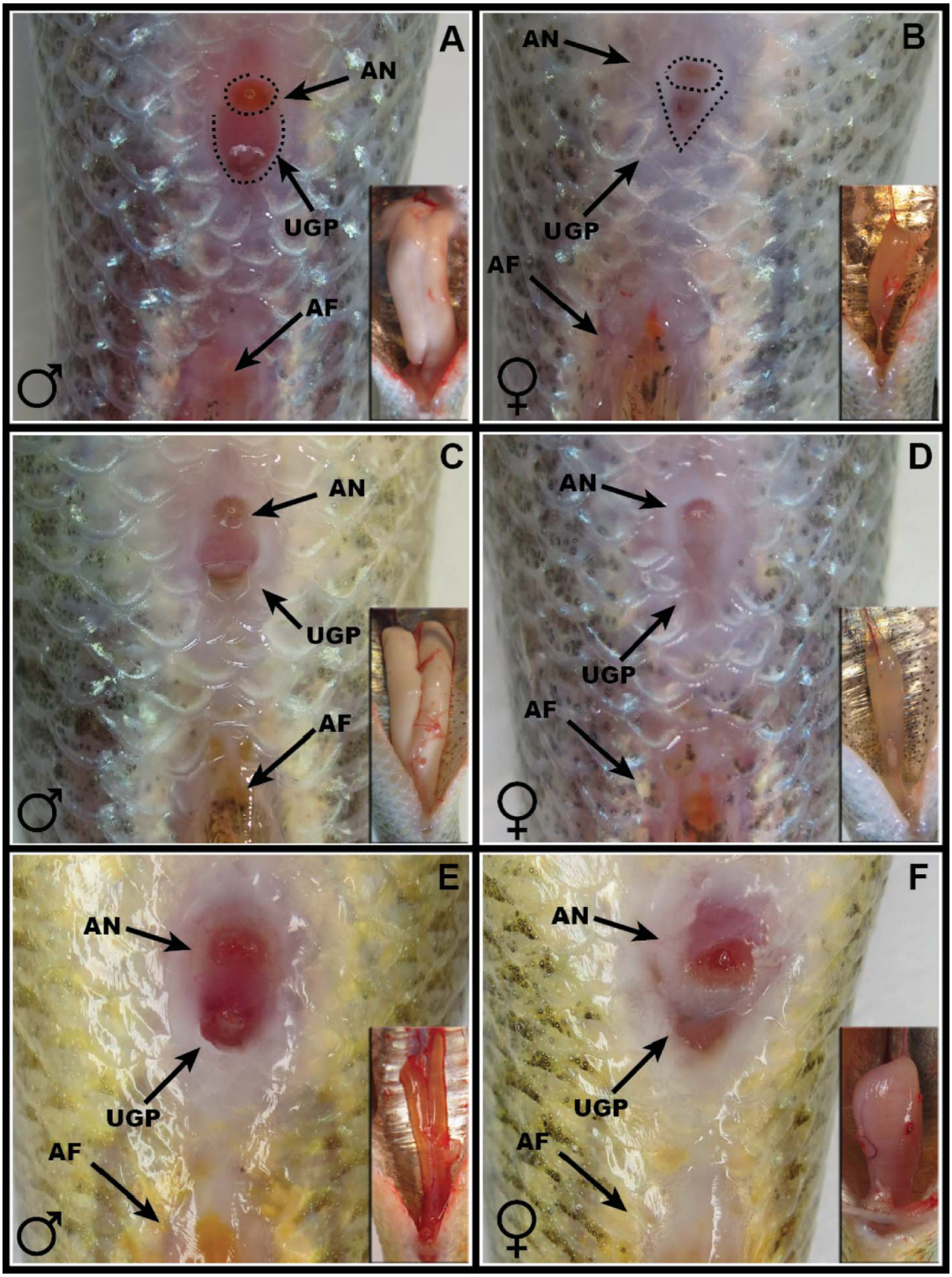

FIGURE 1. External morphology of the urogenital papilla in untreated male and female Yellow Perch from three distinct size-classes. An example of sex confirmation is included in each panel as an inlay. Representative examples were all externally identified correctly. (**A**) Male (♂) Sassafras strain Yellow Perch in size category 1 (80–170 mm). (**B**) Female (♀) Sassafras strain Yellow Perch in size category 1 (80–170 mm). (**C**) Male Sassafras strain Yellow Perch in size category 2 (171–210 mm). (**D**) Female Sassafras strain Yellow Perch in size category 2 (171–210 mm). (**E**) Male Winnebago strain Yellow Perch in size category 3 (211–290 mm). (**F**) Female Winnebago strain Yellow Perch in size category 3 (211–290 mm). Morphological landmarks are indicated in bold block letters as follows: AN = anal opening, UGP = urogenital papilla, AF = anal fin. Dashed lines indicate general shape of the UGP in males (panel A) and females (panel B). Of noticeable contrast between each sex is the rounded UGP in males, the V- or U-shaped UGP in females, darker reddish coloration of the UGP in males versus females, and the UGP generally wider than the AN in males and generally narrower than the AN in females.

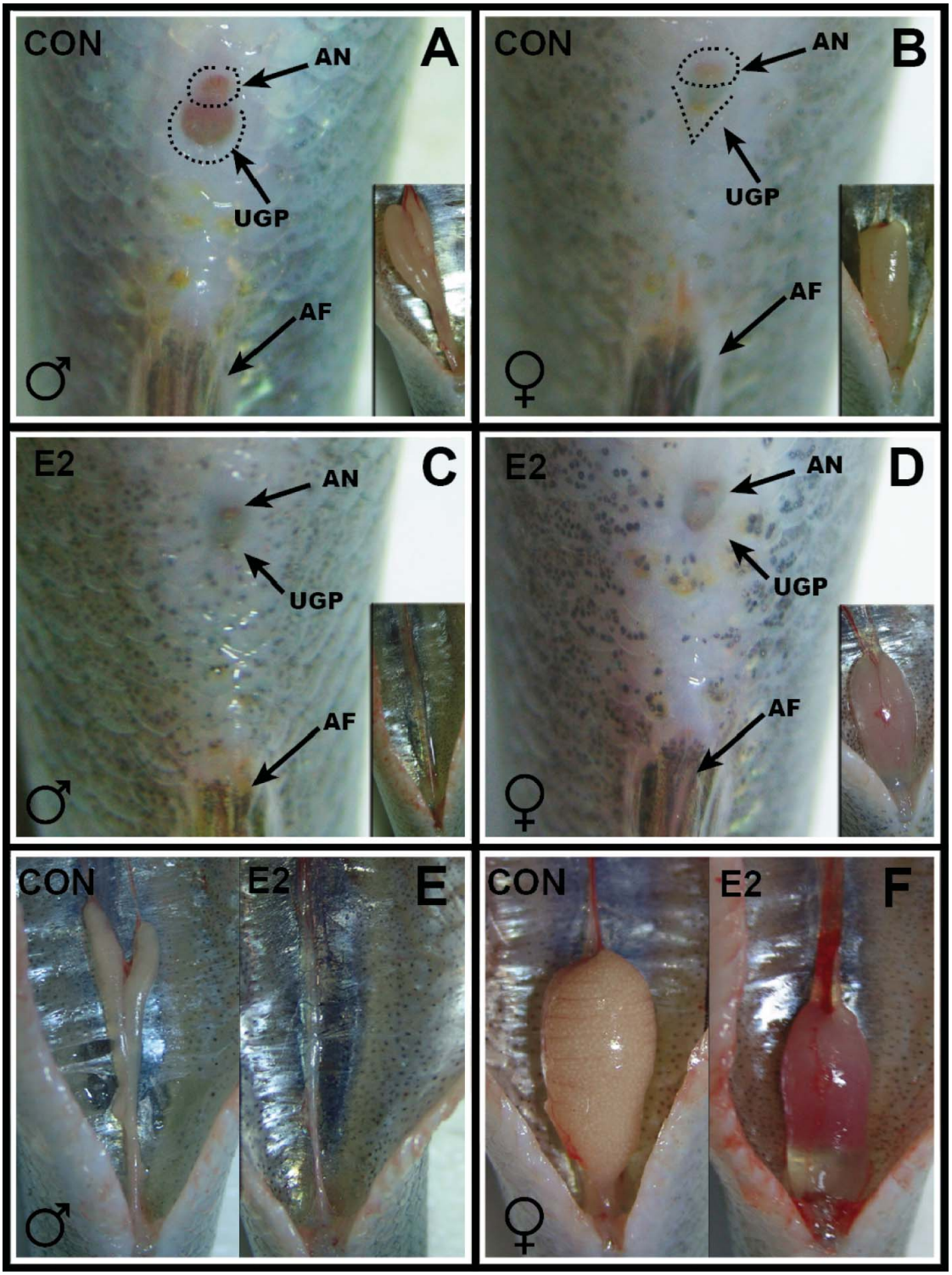

FIGURE 2. External morphology of the urogenital papilla of control and E2-treated male (♂) and female (♀) Yellow Perch. An example of sex confirmation is included in each panel (A–D) as an inlay. Representative examples were all externally identified correctly except the E2-treated male (Choptank and Perquimans strains) in panel C. (**A**) Control male Yellow Perch. (**B**) Control female Yellow Perch. (**C**) E2-treated male Yellow Perch. (**D**) E2-treated female Yellow Perch. (**E**) Internal reproductive organ comparison of control and E2-treated male Yellow Perch. Poor testicular development in the E2-treated males indicates a treatment-induced effect. (**F**) Internal reproductive organ comparison of control and E2-treated female Yellow Perch. It was typical to see hemorrhagic coloration, fluid at the posterior end, and lack of fully formed eggs in the ovaries of E2-treated females. Morphological landmarks are highlighted in bold block letters as follows: AN = anal opening, UGP = urogenital papilla, AF = anal fin. Dashed lines indicate general shape of the UGP in males (panel A) and females (panel B). Of noticeable contrast between each sex is the rounded UGP in males, the V- or U-shaped UGP in females, darker reddish coloration of the UGP in males, and the UGP generally wider than the AN in males and generally narrower than the AN in females. However, E2-treated males externally demonstrated morphological characteristics that more closely resembled those of control females than control males.

TABLE 1. Yellow Perch external sex determination by strain and size. Groups of Yellow Perch from four geographically distinct strains ($n = 1{,}389$) and three distinct size categories were externally scored for sex determination using external morphological criteria. TL range = range of total length in millimeters; mean TL of the group is in parentheses. BW range = range of body weight in grams; mean BW of the group is in parentheses. n = total number of perch in group, (n correct) = total number of perch in group identified correctly, % = percentage [(number of perch identified correctly/total number of perch in identified group) × 100] of perch in group identified correctly, ND = no data.

		Male				Female				Both sexes			
Strain	Size category	TL range (mean)	BW range (mean)	n (n correct)	%	TL range (mean)	BW range (mean)	n (n correct)	%	TL range (mean)	BW range (mean)	n (n correct)	%
Choptank	80–170 mm	105–170 (143.8)	15–74 (40.2)	102 (100)	98.0	81–169 (138.7)	12–93 (39.0)	67 (67)	100.0	81–170 (141.8)	12–93 (39.7)	169 (167)	98.8
	171–210 mm	171–209 (183.1)	56–145 (82.9)	94 (90)	95.7	171–210 (193.0)	63–158 (97.9)	107 (105)	98.1	171–210 (188.4)	56–158 (90.9)	201 (195)	97.0
	211–290 mm	211–285 (234.1)	110–234 (186.0)	181 (179)	98.9	212–266 (240.6)	117–351 (210.3)	59 (59)	100.0	211–285 (235.7)	110–351 (192.0)	240 (238)	99.2
Perquimans	80–170 mm	75–170 (160.7)	40–71 (56.4)	33 (31)	93.9	77–170 (145.2)	57–91 (67.3)	11 (10)	90.9	75–170 (156.8)	40–91 (59.1)	44 (41)	93.2
	171–210 mm	171–210 (186.3)	63–193 (90.3)	140 (130)	92.9	173–210 (192.3)	67–171 (98.3)	103 (100)	97.1	171–210 (188.7)	63–193 (93.4)	247 (230)	93.1
	211–290 mm	212–252 (227.5)	145–263 (196.4)	13 (8)	61.5	211–256 (225.7)	123–320 (190.0)	23 (23)	100.0	211–256 (226.3)	123–320 (192.3)	36 (31)	86.1
Sassafras	80–170 mm	120–170 (156.6)	17–68 (48.7)	77 (76)	98.7	144–170 (158.7)	36–73 (53.0)	44 (44)	100.0	120–170 (157.4)	17–73 (50.3)	121 (120)	99.2
	171–210 mm	171–203 (180.5)	61–105 (76.9)	45 (44)	97.8	172–208 (189.6)	66–132 (97.2)	88 (86)	97.7	171–208 (186.5)	61–132 (90.3)	133 (130)	97.7
	211–290 mm	ND	ND	0 (0)	ND	211–213 (212.0)	136–142 (139.0)	2 (2)	100.0	211–213 (212.0)	136–142 (139.0)	2 (2)	100.0
Winnebago	80–170 mm	ND	ND	0 (0)	ND	ND	ND	0 (0)	ND	ND	ND	0 (0)	ND
	171–210 mm	195–210 (205.1)	81–128 (112.6)	13 (13)	100.0	ND	ND	0 (0)	ND	195–210 (205.1)	81–128 (112.6)	13 (13)	100.0
	211–290 mm	211–237 (219.6)	102–218 (142.2)	41 (38)	92.7	212–270 (244.5)	92–342 (196.9)	146 (146)	100.0	211–270 (239.0)	92–342 (184.9)	187 (184)	98.4
Total		75–285 (197.5)	15–263 (104.4)	739 (709)	95.9	77–270 (192.4)	12–351 (124.1)	650 (642)	98.8	75–285 (195.0)	12–351 (113.5)	1,389 (1,351)	97.3

number of correct external gender identifications and the number of incorrect identifications (97.3% and 2.7%, respectively; binomial proportion test: $Z = 35.23$, $P < 0.0001$), indicating that the technique of external gender identification is very effective in predicting the true gender of Yellow Perch over both sexes, all four strains, and three size categories tested. Despite the single group (Perquimans strain, size category 3) of perch that exhibited lower accuracy in the identification of males only (61.5%), the accuracy of our criteria for determining true gender, as applied to all strains and sizes, was 97.3% for both genders combined (Table 1).

The results of the main effects logistic model showed significant differences in the success of external gender identification between males and females ($P = 0.0028$) for all strains and size categories as well as differences between strains ($P = 0.004$) for both sexes of Yellow Perch and all size categories. No effect of size category on the success of external gender identification was found. Other logistic models showed no significant Strain × Size Category or Strain × True Gender interaction effects. Main effect logistic models were further examined for Strain and True Gender of fish independent of one another. Significant differences for Strain in the success of external gender identification were found for both sexes and all size categories of perch (Wald chi-square = 19.16, df = 3, $P = 0.0003$). The Perquimans strain had statistically lower chances of external gender identification being successful than did the other three strains, which were not different from one another (Table 1). Specifically, odds ratio estimates show that the Choptank strain was 4.2 times as likely as Perquimans strain for external gender identification to be successful (98.3% versus 90.8% across both sexes and all sizes; $P = 0.0003$). Sassafras strain was 4.386 times as likely as Perquimans to be successfully identified externally (98.9% versus 90.8% for both sexes and all sizes; $P = 0.0075$). Winnebago was 4.566 times as likely as Perquimans for external gender identification to be successful (99.2% versus 90.8% for both sexes and all sizes; $P = 0.0149$).

Significant differences for True Gender in the success of external gender identification were found for all strains and size categories of Yellow Perch (Wald chi-square = 9.26, df = 1, $P = 0.0023$), and females were 3.4 times (odds ratio estimate) as likely as males to be successfully identified by external examination ($P = 0.0023$), which corresponds to the reduced frequency of successful identifications (for all strains and size categories) in males (95.9%) versus females (98.8%) (Table 1).

TABLE 2. Yellow Perch external sex determination after treatment with E2, grouped by strain (total $n = 913$). Groups of Yellow Perch were fed either control feed (sprayed with 100% ethanol as the carrier and allowed to evaporate completely) or E2-treated feed (15 mg E2/kg diet, dissolved in the carrier then sprayed on feed and allowed to evaporate completely) for 15 weeks and examined to determine gender using external morphological criteria. TL range = range of total length in millimeters; mean TL of the group is in parentheses. BW range = range of body weight in grams; mean BW of the group is in parentheses. n = total number of perch in group, (n correct) = total number of perch in group identified correctly, % = percentage [(number of perch identified correctly/total number of perch in identified group) × 100] of perch in group identified correctly.

		Choptank				Perquimans				Both strains			
Treatment	Sex	TL range (mean)	BW range (mean)	n (n correct)	%	TL range (mean)	BW range (mean)	n (n correct)	%	TL range (mean)	BW range (mean)	n (n correct)	%
Control	Male	146–200 (174.1)	38–108 (69.5)	131 (125)	95.4	147–210 (180.6)	40–144 (79.3)	156 (148)	94.9	146–210 (174.1)	38–144 (69.5)	287 (273)	95.1
	Female	151–220 (190.1)	49–140 (93.4)	127 (125)	98.4	164–221 (191.4)	57–146 (95.5)	116 (113)	97.4	151–221 (190.1)	49–146 (93.4)	243 (238)	97.9
	Total	146–220 (182.0)	38–140 (81.3)	258 (250)	96.9	147–221 (185.2)	40–146 (86.2)	272 (261)	96.0	146–221 (182.0)	38–146 (81.3)	530 (511)	96.4
E2	Male	139–197 (171.2)	32–92 (59.7)	97 (58)	59.8	145–199 (177.6)	45–96 (70.2)	106 (59)	55.7	139–199 (171.2)	32–96 (59.7)	203 (117)	57.6
	Female	150–215 (184.6)	37–124 (76.6)	101 (63)	62.4	165–217 (192.3)	52–129 (90.9)	79 (52)	65.8	150–217 (184.6)	37–129 (76.6)	180 (115)	63.9
	Total	139–215 (178.1)	32–124 (68.3)	198 (121)	61.1	145–217 (183.9)	45–129 (79.1)	185 (111)	60.0	139–217 (178.1)	32–129 (68.3)	383 (232)	60.6
Grand total		139–220 (180.3)	32–140 (75.7)	456 (371)	81.4	145–221 (184.7)	40–146 (83.3)	457 (372)	81.4	139–221 (180.3)	32–146 (75.7)	913 (743)	81.4

Analysis of External Gender Identification Criteria for Yellow Perch Treated with E2

Data on the accuracy of external gender identification in treated male and female Yellow Perch were obtained from a total of 913 perch for both strains (Choptank and Perquimans) and treatments (control and E2) (Table 2). The results of the three-factor logistic model showed significant main effect differences in the success of external gender identification between males and females (Wald chi-square = 4.12, df = 1, $P = 0.0424$), as well as main effect differences between control and E2-treated perch (Wald chi-square = 106.57, df = 1, $P < 0.0001$). No effect of strain on external gender identification was found, nor were there any significant interactions (Table 2). Odds ratios showed that control-treated Choptank females were 37.7 times more likely to be successfully identified to gender (98.4% versus 62.4%) compared with E2-treated Choptank females (Table 2). Control Perquimans females were 19.6 times more likely to have gender successfully identified (97.4% versus 65.8%) than were E2-treated Perquimans females. Control-treated Choptank males were 14 times more likely to be successfully identified to gender (95.4% versus 59.8%) than were E2-treated Choptank males (Table 2). Control Perquimans males were 14.7 times more likely to have their gender successfully identified (94.9% versus 55.7%) than were E2-treated Perquimans males (Table 2).

A single factor logistic model with Treatment (control and E2) as the main effect was used to determine the odds ratio probabilities for both strains and gender of Yellow Perch. A significant treatment effect was obtained that matched the three-factor logistic model (Wald chi-square = 125.06, df = 1, $P < 0.0001$), and the odds ratio estimate showed that control-treated fish were 17.5 times more likely to be correctly identified to gender (96.4% versus 60.6%) than were the E2-treated fish (Table 2). A single factor logistic model with Gender (male and female) as the main effect was used to determine whether there was a significant gender effect for both of the strains and treatments tested. No significance was obtained. The odds ratio showed that females are 1.3 times more likely to have gender correctly identified than were males, but there was no significant P-value associated with this odds ratio suggesting that there were no real differences in the accuracies for correct gender identification between males (79.6%) and females (83.5%) for the two strains and treatments tested. The finding of this odds ratio pairwise test from the single factor logistic model for true gender contrasts with the overall three-factor logistic model for the control- and E2-treated perch, which shows a significant effect (Wald chi-square = 4.12, df = 1, $P = 0.0424$) of true gender on accuracy of correct gender identification. Furthermore, this finding contrasts with the original main-effects logistic test showing a significant effect (Wald chi-square = 9.26, df = 1, $P = 0.0023$) of gender on accuracy of correct gender identification for the untreated perch (all strains and sizes) shown in Table 1. These differences are probably attributed to (1) the smaller sample size of the data set used for control and E2-treated fish, and (2) the strong (overall) effect that E2 treatment had on reducing accuracy of gender identification in male and female Yellow Perch (Table 2).

The GSIs for the Choptank strain fish were significantly affected by treatment ($F_{1,156} = 56.99$, $P < 0.001$) (control or E2) and sex ($F_{1,156} = 159.06$, $P < 0.001$) (male or female) (two-way ANOVA). The GSI values for control Choptank perch were 1.0 ± 0.05% ($n = 40$) for females and 0.5 ± 0.06% ($n = 40$) for males. The GSI values for E2-treated Choptank fish were 0.75 ± 0.03% ($n = 41$) for females and 0.1 ± 0.03% ($n = 39$) for males, and these values were significantly

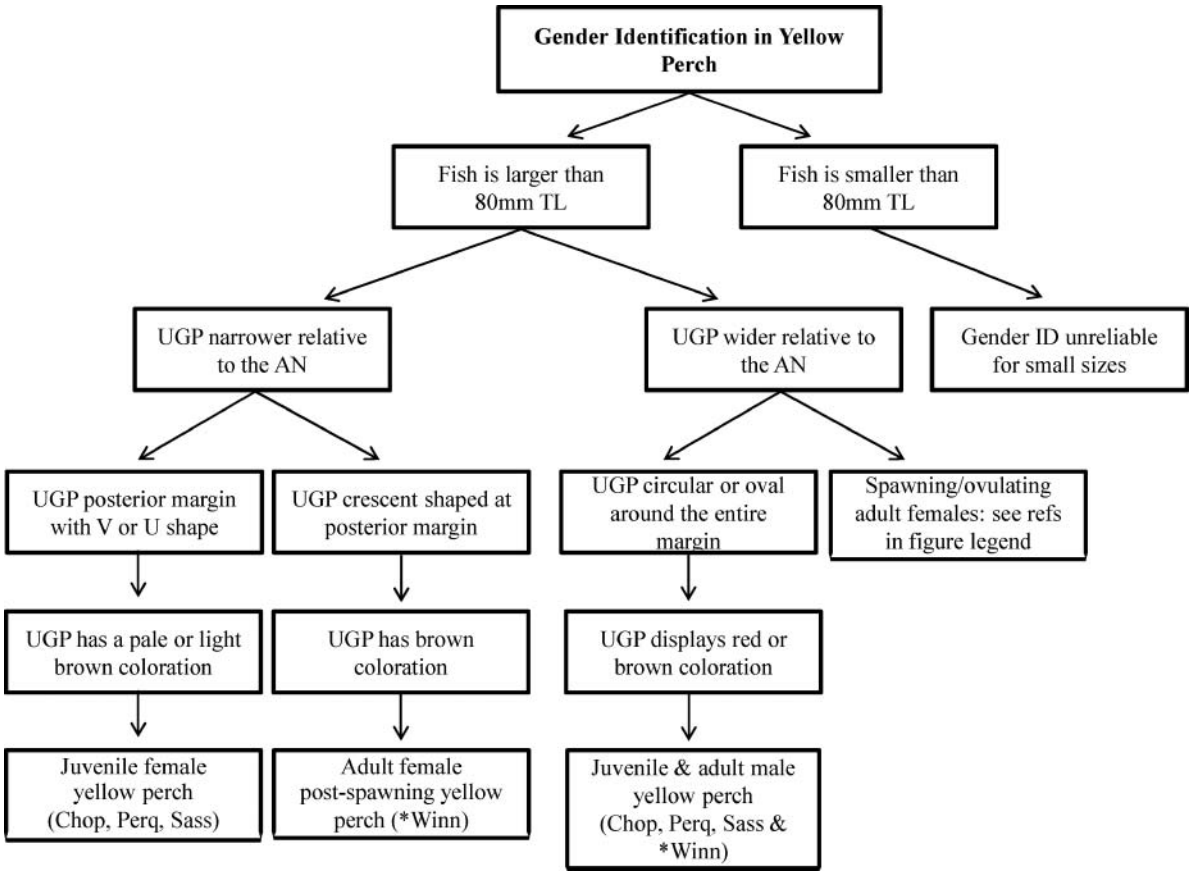

FIGURE 3. Gender identification algorithm for four domesticated geographic strains of Yellow Perch. Abbreviations: AN = anus, UGP = urogenital papilla, Chop = Choptank strain, Perq = Perquimans strain, Sass = Sassafras strain, Winn = Winnebago strain (*Winn indicates males and females had AN swelling that was not present in other strains). See Parker (1942), Hinshaw (2006), and Garling et al. (2007) for images and description of the UGP in spawning and ovulating females.

different from their respective control groups (Tukey–Kramer pairwise comparison: $P < 0.05$). The GSIs for the Perquimans strain fish were significantly affected by treatment ($F_{1, 150} = 46.77$, $P < 0.001$) (control or E2) and sex ($F_{1, 150} = 365.35$, $P < 0.001$) (male or female) (two-way ANOVA), and GSI values for control Perquimans perch were 0.85 ± 0.05% ($n = 34$) for females and 0.19 ± 0.04% ($n = 40$) for males. The GSI values for E2-treated Perquimans fish were 0.58 ± 0.02% for females and 0.03 ± 0.002% ($n = 43$) for males, and these values were significantly different from their respective control groups (Tukey–Kramer pairwise comparison: $P < 0.05$).

Yellow Perch Gender Identification Algorithm

We have developed a decision algorithm for identifying gender of Yellow Perch that uses four primary criteria: (1) total length of the perch, (2) width of the UGP relative to the AN, (3) shape of the UGP, and (4) color of the UGP (Figure 3). This algorithm will assist groups working with Yellow Perch to externally identify gender in their perch populations.

DISCUSSION

A rapid method for external identification of gender in Yellow Perch using differences in morphology and appearance of the external UGP has been developed. This method is highly accurate and can be used to sex juvenile and adult Yellow Perch of four domesticated geographic strains. In the female Yellow Perch we examined, the margin of the UGP typically appeared as a U or V shape that was oriented towards the anal fin, was narrower than the margin of the AN, and displayed either a pale or light brown coloration. In male Yellow Perch, the margin of the UGP was typically round or oval in shape and was wider than that of the AN opening, and coloration ranged from pale brown to a darker red. Using these criteria, differences in the appearance of the external morphology of the female and male Yellow Perch UGP can be detected in fish as small as ~85 mm TL and 15 g body weight. Across all strains and sizes of perch tested, these criteria successfully identified the true gender of male and female Yellow Perch with an accuracy of 97%. Using logistic regression analyses, we can confirm that differences in the accuracy of external gender identification do exist between

geographic strains and sex of the perch, and lower accuracy occurs in the Perquimans strain than in others and in males versus females.

Recently, Malison et al. (2011) reported external morphological criteria that enabled them to identify the gender of a single geographic midwestern strain (from Lakes Mendota and Cherokee in Dane County, Wisconsin; Malison and Held 1992) of Yellow Perch within a size range of 80–280 mm TL (weights were not reported). While our criteria are quantitatively more accurate and can be valid in four geographic strains, the differing criteria do converge but with some exceptions. Specifically, Figures 1 and 2 in Malison et al. (2011) show the female UGP as being crescent shaped and occurring with AN swelling. We only observed females with a crescent-shaped UGP in the Winnebago strain, which was not the typical phenotype observed for females from the other geographic strains (Choptank, Perquimans, or Sassafras) of similar size that were examined in this study. Additionally, we observed AN swelling in both males and females of the Winnebago Strain. By contrast, Malison et al. (2011) reported AN swelling in females only. Consequently, we propose that the crescent-shaped UGP and AN swelling (males and females) are either characteristic of Midwestern strains of Yellow Perch or may be characteristic of sexually mature perch that are in postspawning condition, which was noted for perch of the Winnebago strain in our study. Our findings underscore the differences in UGP morphology between geographic strains and sexual maturity or reproductive history in Yellow Perch. Despite some remaining questions regarding the appearance of the UGP in sexually mature female Yellow Perch across various geographic strains, the appearance of the UGP in spawning females (not shown) is quite distinctive and has been previously documented (Parker 1942; Hinshaw 2006; Garling et al. 2007).

Given all the morphological characteristics identified as necessary to successfully determine gender in Yellow Perch, we developed a decision algorithm (Figure 3). Using the principal features of UGP width relative to AN, UGP color, and UGP shape, we propose a logical framework to enable identification of gender in juvenile and adult male and female Yellow Perch. Given that our criteria have only been developed and validated using four geographic strains of Yellow Perch that were greater than 85 mm TL, we provide the caveat that these identifying characters will need to be tested in perch of varying maturity and from other geographic strains.

To experimentally validate our observational criteria for external identification of gender in Yellow Perch, we treated juvenile Yellow Perch with dietary E2 or a control diet. In both geographic strains treated and examined (Choptank and Perquimans), we found that accuracy of external gender identification was significantly reduced in E2-treated males (57.6%) and females (63.9%) when compared with untreated males (95.1%) and females (97.9%). For E2-treated males, we observed that the margin of the UGP tended to have a U or V shape (indicative of females), lacked distinctive coloration, and appeared equivalent in size to the AN margin, which resulted in the low (57.6%) accuracy for identifying males. A difference in appearance of the UGP in E2-treated and control male Yellow Perch suggests that dietary E2 treatment feminizes the male secondary sexual traits that are characteristic for the UGP of this gender. Interestingly, the region of the UGP in E2-treated females routinely appeared wider than the AN margin and lacked coloration, which caused convergence in gender-specific UGP criteria. Accordingly, convergence of these criteria resulted in a lowered accuracy (63.9%) for identifying the true gender (via external identification) of E2-treated females. Given the difficulties with correctly identifying true gender in E2-treated Yellow Perch and the reduced GSI in these fish, it is likely that E2 treatment delays (via negative feedback) development of primary and secondary sexual characteristics in female Yellow Perch.

It is well known that steroids and steroid antagonists and agonists can affect the reproductive development (primary and secondary sexual characteristics) of gonochoristic finfishes (Sumpter 1997). In this vein, disruption by E2 treatment of the secondary sexual characteristics that enable external identification of gender in Yellow Perch via UGP morphology are consistent with findings of estrogen-induced feminization in other North American species, including Fathead Minnow, Threespine Stickleback *Gasterosteus aculeatus*, and Rainbow Darter *Etheostoma caeruleum* (Hahlbeck et al. 2004; Parrott and Blunt 2005; Elias et al. 2007). Furthermore, the reduced GSI of E2-treated males and females (compared with controls) is consistent with reported results of E2-dependent delayed gonadal development in other finfish species (Scholz and Klüver 2009) and with previous work in Yellow Perch (Malison et al. 1986). While these findings are new for Yellow Perch, the effects of dietary E2 on male and female UGP morphology serves as independent experimental validation of the accuracy of our criteria for using external UGP morphology to identify gender in Yellow Perch. Additionally, the apparent uncoupling of external UGP morphology from actual gender in E2-treated Yellow Perch could indicate exposure to estrogenic substances in settings where endocrine-disrupting chemicals can have an influence.

Access to a robust and cost-effective method for identifying and sorting sexes can have foreseeable benefits to Yellow Perch aquaculture producers and researchers. For aquaculture, the ability to easily recognize gender can enable new producers to collect (without harm to the animal) founder broodstocks from wild populations and enable established producers to manage broodstocks before and during breeding. With respect to aquaculture research, our group is developing genetically improved Yellow Perch broodstocks that exhibit faster growth. In the absence of precise methods for gender identification, it has been necessary to use relaxed selection criteria that require the retention of the upper 40% of performers to allow for a sufficient number of smaller-sized males to develop the next generation of select perch broodstocks (Grzybowski et al. 2010; Rosauer et al. 2011). Now that a more precise method exists for the identification of gender in Yellow Perch, it is possible to implement a bimodal selection regime that focuses on the upper

10–15% of female and male performers, which can further accelerate genetic gain for growth in these domesticated perch strains. For researchers, the ability to easily identify gender in Yellow Perch can enable much-needed, multitank, replicate experiments aimed at studying gender-specific differences in behavior, nutrition, immunology, physiology, and toxicology in this species.

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