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Nonlethal Sex Determination of the Greater Amberjack, with Direct Application to Sex Ratio Analysis of the Gulf of Mexico Stock

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Abstract

Stock assessments for the Gulf of Mexico (Gulf) Greater Amberjack Seriola dumerili continue to designate the stock as overfished and undergoing overfishing, despite increasing regulatory measures. Knowledge of sex-specific spatial distribution and fishing mortality may contribute to our understanding of the stock's overexploitation, especially since Greater Amberjacks may be subject to sex-specific mortality resulting from minimum size regulations. Currently, the sex ratio of the stock is assumed to be 1:1. An average and range of sex ratios were estimated for the Gulf stock based on sampling of fish landed in the recreational and commercial fisheries and based on released fish that were nonlethally sexed during a tagging study of sex-specific movement patterns and release mortality. The nonlethal method of sex determination was developed based on external features of the urogenital region; urogenital catheterization was used to validate the external sexing and to collect oocyte samples for determining maturity stages of females during the spawning season. Of the 238 fish (108 males and 130 females) for which sex was verified, only one smaller female was incorrectly sexed. Urogenital catheterization identified females that were spawning or that would likely spawn in the upcoming spawning season, but no differentiation could be made between immature and resting females. Analysis of published data sets suggested that the Gulf stock has an overall female-skewed sex ratio, with estimates ranging from 1:1.7 to 1:2.5 (male : female), while the nonlethal sexing data from the current study suggest that the overall sex ratio is slightly male skewed (1:0.8) in some regions. All studies report a female-skewed sex ratio of 1:2.3, on average, for 1,000-mm FL and larger fish in the Gulf. Sex ratios that deviate from the assumed 1:1 ratio should be incorporated into future assessments of the Gulf Greater Amberjack stock to investigate potential consequences for stock status and management.

In a given fish species, disproportionate catches of one sex or the other may arise from sex change, sexually dimorphic growth combined with minimum size limits, or regional segregation of sexes. Such disproportionate catches could lead to an alteration of the overall sex ratio, which may impact the population dynamics of the stock due to (1) possible egg or sperm limitation arising from low numbers of mature individuals of a particular sex or (2) shifts in the size or age at maturity of a particular sex (or the size or age at sex change in hermaphroditic species; Huntsman and Schaaf 1994; Armsworth 2001; Alonzo and Mangel 2004, 2005; Heppell et al. 2006; Molloy et al. 2007; Alonzo et al. 2008). For example, age-specific sex ratios are required in the stock assessment for Red Porgy Pagrus pagrus since the sex ratios vary due to protogyny (SEDAR 2012).

The sex and reproductive state of fish sampled during catch surveys are commonly assigned through the use of primary and secondary sexual characters. The most commonly used primary sexual character is the presence and developmental state of either the testes or the ovaries; however, intromittent organs, sex hormones, and sex chromosomes (in reproductive individuals)
greater amberjack may also be used to assign sex. A number of species show a high degree of sexual dimorphism (at least when mature or actively spawning), thus allowing sex to be easily assigned based on secondary sexual characters, such as coloration, size, or other modifications of the body (e.g., nuptial tubercles in many cyprinids, coloration in most labrids and scarids, and the kype in males of many salmonids). However, a number of species, including the greater amberjack *Seriola dumerili*, do not possess obvious sexual dimorphism in secondary sexual characters but may exhibit differences in the urogenital region (e.g., shape and number of urogenital pores). Such differences have been used to successfully assign sex in a number of species (McComish 1968; Casselman 1974; Noltie 1985; Benz and Jacobs 1986; Murie 1991; St-Pierre 1992; Vecsei et al. 2003).

The greater amberjack is a pelagic reef species that is found along the eastern and western Atlantic coasts, in the Mediterranean Sea, and throughout much of the Indian and Pacific oceans. In the western Atlantic Ocean, greater amberjacks are distributed from Nova Scotia to Brazil, including the Caribbean and Gulf of Mexico (hereafter, “Gulf”; Smith-Vaniz 2002). They tend to aggregate around reefs, rocky outcroppings, wrecks, and man-made structures such as oil platforms (Manooch and Potts 1997a, 1997b; Thompson et al. 1999; Harris et al. 2007); this tendency to aggregate may make them susceptible to overfishing (Beasley 1993). In the United States, the greater amberjack is managed as two separate stocks: the U.S. South Atlantic stock and the Gulf stock. Both stocks are subject to commercial and recreational fishing. Concerns about overfishing of the Gulf stock have resulted in increased regulation of the commercial and recreational fisheries since 1990 (Hood 2006), and the most recent assessments of the stock have found it to be overfished and undergoing overfishing (SEDAR 2011, 2014).

Gulf greater amberjack stock assessments (SEDAR 2006, 2011, 2014) have been based on the best available data, but limitations have resulted in the use of surrogate parameters from the U.S. South Atlantic stock and in the use of proxies (e.g., weight at maturity as a proxy for fecundity). Some of these data gaps, such as information on age and growth, have been recently acquired (Murie and Parkyn 2008). Many aspects of reproductive biology critical to understanding the sustainability of the Gulf stock are lacking, such as sex ratio estimates. The sex ratio of greater amberjacks in the Gulf is currently unknown and has therefore been assumed to be 1:1 in stock assessments (SEDAR 2006, 2011, 2014). However, it is not known how deviations from this sex ratio might influence the population dynamics of the greater amberjack. If greater amberjacks demonstrate regional segregation by sex, as was suggested by Thompson et al. (1999), this may result in regional skewing of sex ratios and hence the disproportionate representation of one sex or the other in catches from a particular region. There is also the potential for a disproportionate representation of females in the harvested catch due to the faster growth of females than males (Harris et al. 2007; Murie and Parkyn 2008) and due to the minimum length limits placed on the fisheries (i.e., sex selectivity by the fishery).

Obtaining data on the sex of greater amberjacks landed in the commercial and recreational fisheries may be difficult and potentially biased. In the commercial fishery, fish are generally brought to port gutted, making it impossible to sex the fish by examining the gonads. In addition, port sampling of the recreational fishery sector generally only samples a small portion of the landed catch, which may represent only a small fraction of the total catch due to the large minimum length limit (762 mm FL), restricted bag limit (1 fish-person$^{-1}$·d$^{-1}$), and voluntary release of fish (i.e., released or discarded fish are rarely sampled). The development of a nonlethal sexing method for greater amberjacks provides an alternative means of estimating the sex ratio. Such a method could be applied in the field by researchers or by onboard fishery observers to determine the sex of the entire catch, including releases and discards, rather than simply obtaining sex information via sampling a fraction of the landed catch.

Analysis of sex chromosomes from genetic samples is a nonlethal technique for determining sex in some species, but cyogenetically differentiated sex chromosomes appear to be rare among marine teleosts (Galetti et al. 2000). The greater amberjack is among those species for which sex chromosomes have not been identified (Sola et al. 1997). Other nonlethal methods have also been developed to assess sex and maturity in a number of other fish species (see Smith 2011). However, to apply a nonlethal sex determination method in the field, it should be applicable throughout the year and over a range of sizes; furthermore, the method should not require anesthesia since greater amberjacks are harvested for human consumption (Coyle et al. 2004; Kahn and Mohed 2010). Methods that require anesthesia would also be impractical to perform on large fish, such as greater amberjacks, while at sea on commercial and recreational fishing vessels. In addition, the nonlethal sexing method should be relatively simple and quick to perform as well as minimally invasive, allowing individuals to be released in good health; minimal cost requirements for the method would also be desirable. Based on these criteria, nonlethal sexing through the examination of external urogenital features appeared to be a potentially viable method for application to the greater amberjack. Assessment of external urogenital features cannot, however, be used to obtain the maturational status of an individual. Urogenital catheterization meets all of the criteria outlined above but can also potentially provide the maturation status of an individual as well as validation of other sexing methods (e.g., the use of external urogenital features) through the collection of gonadal tissues or fluids (Smith 2011).

The goal of this study was to determine whether the combination of using external urogenital features and urogenital catheterization would allow for the nonlethal determination of sex in the greater amberjack and the relative maturation status of females. Application of this method, in combination with analysis of prior studies and published data, was then used to
develop a range of estimates for the overall sex ratio of the Gulf Greater Amberjack stock.

METHODS

Sex differentiation of urogenital pores.—Initially, eight Greater Amberjacks (6 males and 2 females) were collected as part of a tagging study (Murie et al. 2013) and were sacrificed to allow examination of their urogenital regions for the presence of morphological differences in the urogenital pores and surrounding tissues. These eight individuals were collected from November 2008 through January 2009, approximately 2–4 months prior to the onset of the peak spawning season (March–April) for the Greater Amberjack in the Gulf (Wells and Rooker 2004; Murie and Parkyn 2008). This helped to ensure that differences observed between males and females were not limited to the spawning season and the periods immediately prior to and after peak spawning. Examination consisted of using a blunt probe to locate the anus and urogenital pore(s). Differences in the spacing, location, and general appearance of the urogenital pore(s) and surrounding tissues were noted. Similar observations were made on three individuals (1 male and 2 females) to ensure that no differentiating characters had been overlooked; these three fish were sexed in the field during the first application of the nonlethal sexing method and were sacrificed for validation.

Field-based sex identification by examination of urogenital pores and the accuracy of sex determination.—To apply the external sexing method to fish sampled in the field and to determine the accuracy of the method, Greater Amberjacks were sexed during tagging trips from 2009 to 2012. Fish were caught with hook-and-line fishing gear off the coast of Louisiana, the Gulf coast of Florida, and the Florida Keys. Fish were measured for FL (nearest mm) and tagged below the anterior portion of the second dorsal fin with a Hallprint dart tag; two pectoral fin rays were removed for aging and genetic analyses as part of the tagging study. Fish were then sexed by examining the external features of the urogenital region. To do this, a blunt probe was used to find both the genital pore and the urinary pore; using the sex differentiation criteria developed earlier, the fish was then scored as male or female based on (1) the location of each pore in relation to the other and (2) the appearance of the pores and surrounding tissue. The accuracy of sex determination with this method was based on validation obtained through urogenital catheterization, the expression of milt on insertion of a blunt probe into the genital pore or through abdominal pressure, and dissection of sacrificed individuals. Captured fish with oocytes extruded out the genital pore or with freely flowing milt were not used to determine the accuracy of the external sexing method. Sexing and catheterization of each fish were performed while the fish was placed on its side on a measuring board.

Urogenital catheterization was attempted on all females that appeared to be reproductively active (i.e., those in which the genital pore was enlarged; Figure 1C) and was attempted randomly on males (that did not express milt) and females of various lengths. The catheter consisted of a 3-mL Luer-Lok-tip disposable syringe and plastic microbore tubing with the following specifications: an inner diameter of 0.76 mm, an outer diameter of 2.23 mm, a wall thickness of 0.76 mm, and a length of approximately 20 cm. The diameter of the tubing was selected based on the size of the genital pore and the maximum diameter of the oocytes. The tubing was attached to the syringe via a 1.6-mm (0.0625-in) inner diameter, female Luer-thread style to 500 series barb adaptor. The catheter was gently inserted into the genital pore as far as possible and then was slowly removed while applying suction with the syringe. The distance for which the tubing could be inserted depended on the length and reproductive status of the fish. In general, the tubing was inserted approximately 4–8 cm in smaller fish (<800 mm FL), whereas the tubing could be inserted farther (8–12 cm or more) in larger fish. Using this catheterization procedure, milt samples were also obtained from males that did not express milt with abdominal pressure. All samples obtained by catheterization were placed in 20-mL scintillation vials containing 5 mL of a 10% solution of phosphate-buffered formalin. Between each use of the catheter, it was flushed with deionized water until clean.

Among the fish that did not express milt and that did not yield a sample from catheterization, a subsample of individuals was sacrificed for validation of the sex determination. These sacrificed fish were initially sexed in the field based only on

FIGURE 1. Urogenital region of the Greater Amberjack, with anus, genital pore, and urinary pore denoted: (A) male; (B) female; and (C) reproductively active female. The urinary pore is the posterior-most structure. Scale bar in all images is 5 mm.
TABLE 1. Maturation stages of Greater Amberjack females based on the general appearance of oocytes from catheter samples; stages follow the descriptions provided by Grau et al. (1996), Micale et al. (1999), Poortenaar et al. (2001), and Harris et al. (2004, 2007).

<table>
<thead>
<tr>
<th>Maturation stage</th>
<th>Defining oocyte type</th>
<th>Oocyte stages present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature/resting</td>
<td>Primary growth</td>
<td>Stages up to late perinucleolus stage</td>
</tr>
<tr>
<td>Early developing</td>
<td>Early developing</td>
<td>Stages up to cortical alveolus stage</td>
</tr>
<tr>
<td>Late developing</td>
<td>Late developing</td>
<td>Stages up to yolk granule</td>
</tr>
<tr>
<td>Ripe</td>
<td>Hydrated or late developing and degraded</td>
<td>Stages up to yolk granule and hydrated and/or degraded oocytes</td>
</tr>
<tr>
<td>Spent</td>
<td>Early developing and degraded</td>
<td>Stages up to cortical alveolus stage and degraded oocytes, but no yolk granule or hydrated oocytes</td>
</tr>
</tbody>
</table>

the appearance of the urogenital area. In the laboratory, a colleague that was not involved with the sex determination project prepared each of these fish by wiping the fish’s urogenital area clean to remove any expelled reproductive material and waste. Each fish was then re-sexed without a priori knowledge of its identification or its initial sex as determined in the field. The actual sex of the fish was then determined by direct visual inspection of its gonads.

*Maturation staging of females via urogenital catheterization.*—To investigate the maturation status of females that had been catheterized, the oocyte samples were viewed under a dissection microscope at 10–50 × depending on the size of the oocytes. A Motic imaging system was used to measure the diameter of 50 oocytes or as many as possible if fewer than 50 measurable oocytes had been extracted via catheterization. All of the hydrated oocytes were measured; atretic (degraded) oocytes were not measured, but their presence was noted. Measured oocytes were classified based on their size and general appearance, and the types of oocyte present were used to classify the maturation stage (Table 1) of individual females that had been catheterized. Immature or resting females contained only primary growth oocytes (up to late perinucleolus stage); early developing females had early developing oocytes (late perinucleolus stage up to cortical alveolus stage) present; late developing females contained late developing oocytes (lipid granule stages); ripe females had hydrated oocytes present or had late developing and degraded oocytes co-occurring; and spent females had early developing and degraded oocytes co-occurring without the presence of late developing oocytes (Grau et al. 1996; Micale et al. 1999; Poortenaar et al. 2001; Harris et al. 2004, 2007). The size frequencies of oocytes in various stages were plotted and compared with ranges given by Grau et al. (1996), Micale et al. (1999), and Harris et al. (2007). No differentiation could be made between immature and resting females, as this differentiation is based mainly on smaller oocyte stages that are not easily extracted with catheters, on differences in the thickness of the ovarian wall, and on the presence of muscle bundles in the ovarian lamellae (Grau et al. 1996; Mackie 2000; Harris et al. 2004, 2007). The number of fish classified in each maturation stage was calculated for each 100-mm length-class and for each month in which catheter samples were collected.

*Sex ratio determination.*—Sex ratios of the Greater Amberjack in the Gulf were determined based on data sets from fisheries sampling and the published literature as well as by applying the alternative, nonlethal sexing methods to fish collected in field-based sampling. A data set used in an age, growth, and reproduction study of the Gulf Greater Amberjack stock by Murie and Parkyn (2008) was analyzed for estimates of overall sex ratio. Murie and Parkyn (2008) determined sex by direct observation of the gonads for over 1,600 individuals collected from commercial and recreational landings and fisheries-independent sampling. In addition, sex ratios were estimated for several length-classes: (1) fish smaller than 700 mm FL, representing those close to or below the recreational length limit during the period of the Murie and Parkyn (2008) study; and (2) all 700-mm FL and larger fish, encompassing those vulnerable to recreational fishing (≥711 mm FL during 1990–2008; ≥762 mm FL during 2009–present) and commercial fishing (≥914 mm FL). Sex ratios were also analyzed for 1,000-mm FL and larger fish as a separate group because previous studies have indicated that Greater Amberjacks over 1 m in length are predominantly females (Beasley 1993; Thompson et al. 1999; Harris et al. 2007). Chi-square tests were performed on the overall sex ratio and on the sex ratio for each length-class to determine if the ratios differed significantly from 1:1, the value currently assumed in Greater Amberjack stock assessments (SEDAR 2006, 2011). Annual sex ratio estimates from the Murie and Parkyn (2008) data set were restricted to 2002–2008 because sample sizes in years prior to 2002 were low (<50 fish/year). The annual sex ratios were calculated to estimate the range of observed sex ratios in addition to an overall sex ratio estimated for all years combined. Sex ratios were also calculated in the same manner for fish that were nonlethally sexed in conjunction with the tag-and-release study conducted by Murie et al. (2013) in the Gulf and off the Florida Keys. Sampling was conducted aboard commercial, recreational, and research vessels. Fish that were nonlethally sexed from waters off the Florida Keys or that were caught on sampling trips in which only a portion of the catch was sexed were not included in the sex ratio analysis. Details
TABLE 2. Overall sex ratios (male : female) of Greater Amberjacks and sex ratios for three length-classes (<700 mm, ≥700 mm, and ≥1,000 mm FL) based on data from studies in the Gulf of Mexico (Gulf) and the U.S. South Atlantic.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex ratio</th>
<th>n</th>
<th>χ²</th>
<th>P</th>
<th>Years analyzed</th>
<th>Regions sampled</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>All fish</td>
<td>1:2.5</td>
<td>351</td>
<td>64.96</td>
<td>&lt;0.001</td>
<td>1989–1992</td>
<td>Louisiana Florida (Gulf coast), Alabama, Louisiana</td>
<td>Thompson et al. 1999</td>
</tr>
<tr>
<td></td>
<td>1:1.69</td>
<td>1,526</td>
<td>103.80</td>
<td>&lt;0.001</td>
<td>2002–2008</td>
<td>Florida (Gulf coast), Alabama, Louisiana</td>
<td>Murie and Parkyn 2008 data set (this study)</td>
</tr>
<tr>
<td></td>
<td>1:0.95</td>
<td>328</td>
<td>0.20</td>
<td>0.659</td>
<td>2009–2012</td>
<td>Florida (Gulf coast), Alabama, Louisiana</td>
<td>Nonlethal sexing (this study)</td>
</tr>
<tr>
<td></td>
<td>1:1.11</td>
<td>2,206</td>
<td>5.89</td>
<td>0.015</td>
<td>2000–2004</td>
<td>U.S. South Atlantic</td>
<td>Harris et al. 2007</td>
</tr>
<tr>
<td>Fish &lt; 700 mm FL</td>
<td>1:1.39</td>
<td>293</td>
<td>7.54</td>
<td>0.006</td>
<td>2002–2008</td>
<td>Florida (Gulf coast), Alabama, Louisiana</td>
<td>Murie and Parkyn 2008 data set (this study)</td>
</tr>
<tr>
<td></td>
<td>1:0.85</td>
<td>50</td>
<td>0.32</td>
<td>0.571</td>
<td>2009–2012</td>
<td>Florida (Gulf coast), Alabama, Louisiana</td>
<td>Nonlethal sexing (this study)</td>
</tr>
<tr>
<td></td>
<td>1:0.29</td>
<td>22</td>
<td>na*</td>
<td>na*</td>
<td>2000–2004</td>
<td>U.S. South Atlantic</td>
<td>Harris et al. 2007</td>
</tr>
<tr>
<td>Fish ≥ 700 mm FL</td>
<td>1:1.79</td>
<td>1,233</td>
<td>99.92</td>
<td>&lt;0.001</td>
<td>2002–2008</td>
<td>Florida (Gulf coast), Alabama, Louisiana</td>
<td>Murie and Parkyn 2008 data set (this study)</td>
</tr>
<tr>
<td></td>
<td>1:0.97</td>
<td>278</td>
<td>0.20</td>
<td>0.659</td>
<td>2009–2012</td>
<td>Florida (Gulf coast), Alabama, Louisiana</td>
<td>Nonlethal sexing (this study)</td>
</tr>
<tr>
<td></td>
<td>1:1.12</td>
<td>2,185</td>
<td>7.38</td>
<td>0.007</td>
<td>2000–2004</td>
<td>U.S. South Atlantic</td>
<td>Harris et al. 2007</td>
</tr>
<tr>
<td>Only fish ≥ 1,000 mm FL</td>
<td>1:2.56</td>
<td>173</td>
<td>32.51</td>
<td>&lt;0.001</td>
<td>1989–1992</td>
<td>Louisiana</td>
<td>Beasley 1993; Thompson et al. 1999</td>
</tr>
<tr>
<td></td>
<td>1:2.13</td>
<td>202</td>
<td>21.56</td>
<td>&lt;0.001</td>
<td>2002–2008</td>
<td>Florida (Gulf coast), Alabama, Louisiana</td>
<td>Murie and Parkyn 2008 data set (this study)</td>
</tr>
<tr>
<td></td>
<td>1:2.18</td>
<td>35</td>
<td>4.83</td>
<td>0.03</td>
<td>2009–2010</td>
<td>Florida (Gulf coast), Alabama, Louisiana</td>
<td>Nonlethal sexing (this study)</td>
</tr>
<tr>
<td></td>
<td>1:1.96</td>
<td>584</td>
<td>92.74</td>
<td>&lt;0.001</td>
<td>2000–2004</td>
<td>U.S. South Atlantic</td>
<td>Harris et al. 2007</td>
</tr>
</tbody>
</table>

A chi-square test was not performed due to the low sample size in this category.

regarding the years of data analyzed and the spatial regions sampled are summarized in Table 2.

RESULTS

Sex Differentiation by Examination of Urogenital Pores

Urogenital pores of male and female Greater Amberjacks were surrounded by white, papilla-like folds of tissue (Figure 1). In addition, both males and females were found to have separate urinary and genital pores. However, the positions of these pores in relation to one another were different. In males, the genital pore was positioned along the midline, with the urinary pore located directly posterior to it. The two pores were separated from one another by a thin (generally ≤1 mm), flesh-colored septum (Figure 1A). The septum dividing the two pores extended across the urinary pore, and upon insertion of a probe into the urinary pore, it generally covered the genital pore and vice versa, making it difficult to observe both pores at one time. In females, the genital and urinary pores were both situated along the midline, or one pore was positioned along the midline and the other was positioned slightly off-center. The two pores in females were separated by a greater distance than in males (generally >1 mm); in most cases, the tissue between the pores was at least partially white in color (Figure 1B). In some cases, the white, papilla-like folds of tissue that surrounded the urogenital pores extended between the two pores in females. The greater separation of the pores in females allowed for easier viewing of both pores simultaneously—even upon insertion of a probe—than was possible when males were examined. Observation of live, mature females in spawning condition revealed that the genital pore in these individuals was much larger than that in males and was often crescent shaped (Figure 1C).

Field-Based Sex Identification by Examination of Urogenital Pores and the Accuracy of Sex Determination

Overall, 553 Greater Amberjacks were sexed in the field via characters associated with the urogenital pores (258 males and
294 females). Of these fish, verification of sex was obtained for 238 individuals (108 males and 130 females). Verification was obtained mainly via expression of milt in males and via catheterization in females (Figure 2).

In total, 237 fish were sexed correctly, yielding an overall accuracy of 99.6%. All of the males \( (n = 108) \) and 99.2% of the females \( (n = 130) \) of all lengths (534–1,412 mm FL) were accurately sexed (Table 3). The one exception was a 636-mm FL female that was incorrectly sexed in the field; this fish was sacrificed and sexed correctly later in the laboratory based on urogenital pore characteristics (prior to direct observation of her gonads via dissection).

**Maturation Staging of Females via Urogenital Catheterization**

The majority (95 of 97) of the catheter samples used for maturation staging of females were collected during March–May. All stages of maturation were observed in these catheter samples, including females with oocytes classified as immature or resting (Figure 3A), in stages of early development (Figure 3B), in stages of late development or ripe and spawning (Figure 3C), and spent (Figure 3D). Of the 97 catheter samples obtained from females, 92 could be staged according to the criteria outlined in Table 3.

![Figure 2](https://bioone.org/journals/Marine-and-Coastal-Fisheries-Dynamics-Management-and-Ecosystem-Science-2019-10.1080-30707611.2018.1546034-fig2)

**FIGURE 2.** Number of Greater Amberjacks that were nonlethally sexed and number of fish for which sex was verified by milt expression, urogenital catheterization, or dissection.

![Figure 3](https://bioone.org/journals/Marine-and-Coastal-Fisheries-Dynamics-Management-and-Ecosystem-Science-2019-10.1080-30707611.2018.1546034-fig3)

**FIGURE 3.** Representative images of oocytes at various stages of maturity collected via urogenital catheterization of Greater Amberjack females: (A) female classified as immature or resting (only primary growth oocytes \( P \) are visible); (B) female classified as early developing (ED; oocytes up to the cortical alveolus stage are present); (C) female classified as ripe (fully hydrated oocytes \( H \) are present; yolk granule stages [late developing, LD] are also present); and (D) female classified as spent (with degraded oocytes \( D \) but no yolk granule or hydrated oocyte stages). Scale bar in all images is 0.5 mm.
TABLE 3. Percentage of Greater Amberjacks that were correctly sexed by examination of urogenital pores during each month.

<table>
<thead>
<tr>
<th>Month</th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% correct</td>
<td>n</td>
<td>% correct</td>
</tr>
<tr>
<td>Mar</td>
<td>28</td>
<td>96.4</td>
<td>15</td>
<td>100.0</td>
</tr>
<tr>
<td>Apr</td>
<td>52</td>
<td>100.0</td>
<td>46</td>
<td>100.0</td>
</tr>
<tr>
<td>May</td>
<td>23</td>
<td>100.0</td>
<td>42</td>
<td>100.0</td>
</tr>
<tr>
<td>Jun</td>
<td>3</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jul</td>
<td>23</td>
<td>100.0</td>
<td>5</td>
<td>100.0</td>
</tr>
<tr>
<td>Nov</td>
<td>1</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>99.2</td>
<td>108</td>
<td>100.0</td>
</tr>
</tbody>
</table>

in Table 1. Catheterized females ranged in length from 534 to 1,412 mm FL (Table 4), and the maturity stages of early development and late development could be differentiated in females as small as 800 mm FL (Table 4). In addition, a number of females larger than 800 mm FL that were collected during the peak of the spawning season (March–May) could be identified as actively spawning (ripe) based on the presence of hydrated oocytes or the co-occurrence of lipid granule stage oocytes and degraded oocytes from a prior spawning event (Figure 4; Table 4).

The mean diameter of measured oocytes showed distinct separation in the sizes of each oocyte category used to determine the maturation status of catheterized females (Figure 5). The size separation in oocyte categories indicated accurate classification in determining female maturation status.

Catheter samples from five fish did not contain visible oocytes when examined at magnifications up to 50×. However, the tissue obtained from these five fish did not resemble milt in color or texture but did resemble tissue surrounding oocytes from other samples, both in color and texture. At higher magnification levels (up to 100×), some structures that loosely resembled oocytes were visible. These samples were all relatively small and probably came from immature or resting females.

Sex Ratio Determination

The overall sex ratio estimate for the Gulf Greater Amberjack stock based on nonlethal sexing was not different from 1:1 ($\chi^2 = 0.20, df = 1, P = 0.659$) but was significantly female skewed (1:1.7, male : female; $\chi^2 = 103.80, df = 1, P < 0.001$) based on fishery-dependent and fishery-independent sampling (Murie and Parkyn [2008] data set; Table 2). Yearly sex ratio estimates from 2002 to 2008 based on the Murie and Parkyn (2008) data set averaged 1:1.8 ± 0.14 (mean ± SE; n = 7).

Based on the Murie and Parkyn (2008) data set, sex ratio estimates for sublegal-sized fish (<700 mm FL), legal-sized fish (≥700 mm FL, including fish ≥1,000 mm), and all fish combined were similar and significantly female skewed (Table 2). For the nonlethal data set, estimates of sex ratio for the two size-groups and the overall sex ratio for all fish pooled were also similar but did not significantly differ from 1:1 (Table 2).

Sex ratio estimates for 1,000-mm FL and larger Greater Amberjacks in the Gulf were significantly female skewed based on the nonlethal sexing data set (1:2.1, male : female) and the Murie and Parkyn (2008) data set (1:2.2; Table 2). Overall, the

TABLE 4. Number of catheterized Greater Amberjack females that were classified into each maturation stage (described in Table 1) by 100-mm length-class.

<table>
<thead>
<tr>
<th>Maturation stage</th>
<th>Length-class (mm FL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500</td>
</tr>
<tr>
<td>Immature/resting</td>
<td>21</td>
</tr>
<tr>
<td>Early developing</td>
<td>5</td>
</tr>
<tr>
<td>Late developing</td>
<td>23</td>
</tr>
<tr>
<td>Ripe/running</td>
<td>42</td>
</tr>
<tr>
<td>Spent</td>
<td>1</td>
</tr>
</tbody>
</table>
average sex ratio for 1,000-mm FL and larger fish in the Gulf (based on all values in Table 2) was 1:2.3 ± 0.13 (n = 4).

DISCUSSION

Urogenital characters used to distinguish between the sexes of Greater Amberjack become enhanced during the spawning season (e.g., the enlarged genital pore in females), which makes sex determination for actively spawning fish easier than that for nonspawning fish. However, other fish sampled during the peak spawning period were either immature females or resting mature females (i.e., those that skipped spawning), and they did not display the overt characters apparent in actively spawning fish. Along with fish that were sampled outside of the peak spawning season, all of these fish were sexed with a high degree of accuracy (99% and 100% for females and males, respectively) that was similar to or greater than the levels of accuracy reported in previous reproductive studies of the Greater Amberjack.

Despite the general applicability of examining urogenital features to nonlethally sex the Greater Amberjack, one potential limitation of the method was that it could not easily be applied to fish smaller than 500 mm FL because their urogenital pores were too small to easily locate in live fish. The use of a magnifying glass may improve application of the method to these smaller individuals, but it may be that the differences observed in larger fish have not fully developed in smaller fish. Applying a dye, such as methylene blue, to the urogenital region can improve the sexing of some species (Rakocy and McGinty 1989; Popma and Masser 1999), but for those species the difference is in the number of pores in each sex and not the position of the pores relative to one another. The small size of urogenital pores may also contribute to incorrect sexing of fish between 500 and 700 mm FL; there is a tendency to misidentify immature females in this length range as males because pores in these females are smaller and have less separation than those in larger females or in mature females within the 500–700-mm length range. This potential problem was exemplified by the single, small (636-mm FL) female that was incorrectly sexed in the field as a male during one of the first applications of the external sexing method on live individuals; the fish was later successfully identified as a female in the laboratory prior to dissection. With the exception of this female, the method of sexing Greater Amberjacks by means of urogenital characteristics was accurate for fish larger than 500 mm FL, regardless of sex or length.

The sex determination method used for the Greater Amberjack in the present study was adapted from existing methods applied to other species (McComish 1968; Casselman 1974); therefore, this general approach could likely be applied to other species that are sexually dimorphic with respect to urogenital pores. For example, the method could easily be adapted to other *Seriola* spp. in the Gulf and elsewhere in the world. One relatively large female of the closely related Almaco Jack *S. rivoliana* was captured during this study and had the same urogenital features exhibited by Greater Amberjacks. In many cases, the differences in the urogenital regions between the sexes may be minor, especially in fish that are not actively spawning. It may therefore take careful examination of sacrificed individuals to detect such differences, but once these small differences are identified, sexing can still be highly accurate, as was the case for the Greater Amberjack.

The general maturation stage of mature Greater Amberjack females was easily obtained in the field by examining oocyte samples that were extracted via urogenital catheterization in the months during and immediately after the peak spawning season (March–May). Kožul et al. (2001) and Mylonas et al. (2004) previously used urogenital catheterization of Greater Amberjacks to monitor egg maturation during captive spawning, but it is encouraging that this method can be applied under field conditions as well. Lines of evidence indicating that the classification of an individual to a particular maturation stage was accurate included (1) the distinct separation in mean diameter among the oocyte types measured in this study and (2) the similarity between the oocyte diameters reported here and those reported in previous reproductive studies of the Greater Amberjack (Grau et al. 1996; Micale et al. 1999; Harris et al. 2007). Use of nonlethal catheterization for staging Greater Amberjack females was therefore a relatively simple method that required very little (and inexpensive) equipment, yet it proved to be very accurate.
on the appearance of other characters in addition to the oocyte type, such as the width of the ovarian wall and the presence of muscle bundles (Brown-Peterson et al. 2011). It is not possible to observe these features with the use of a catheter alone. Some fish that were assigned a particular maturation stage could also have harbored more advanced oocytes that were not collected via the catheter, as catheter samples from live fish were not compared with gonad samples from the same individuals post-mortem. However, previous studies of different species have shown that catheter samples generally agree with gonad samples from the same individuals (Shehadeh et al. 1973; Garcia 1989; Alvarez-Lajanchoire et al. 2001; Ferraz et al. 2004).

Nonlethal sexing of the Greater Amberjack and other fishes can have a variety of useful applications. For example, the celerity of the urogenital sexing method and the catheterization method (<1 min/fish in most cases), their simplicity, and the minimal training required make them suitable candidates for use by on-board fisheries observers to obtain sex and maturation data, as on-board observers generally require methods that allow for relatively rapid data collection (G. Fitzhugh, National Marine Fisheries Service, personal communication). This would allow estimates of sex ratios based on the entire catch, not just the landed catch, since sex could be determined before fish are discarded due to regulations. These sex ratios may better reflect the true sex ratio of the population since they would not be biased by minimum size limits. It would still be important to monitor the sex ratio of the landed catch to determine whether one sex is disproportionately harvested compared with the other in relation to their relative proportions in the population.

Another application of the nonlethal sexing method would be in tag-and-release studies, such as the Murie et al. (2013) study of the Gulf Greater Amberjack stock. The present study was conducted as part of that tag-and-release study, and the resulting nonlethal data on sex are currently being used to elucidate information on sex-specific migration patterns, growth rates, and mortality rates as tagged fish are recaptured. Tagging studies of other species would also benefit from information on the sex of released fish, as such data are generally unavailable due to the paucity of tag returns with accompanying sex information and the potential for misidentification by those who have recaptured the fish (St-Pierre 1992).

Port samplers monitoring fish from commercial vessels could also take advantage of urogenital features for sexing fish because most of the commercial catch is landed in a gutted condition (i.e., no gonads). Port samplers could use the urogenital features to assign sex to the samples (as long as the urogenital region is not damaged during gutting), which would be beneficial since sex is unknown for most of the Greater Amberjack landed in commercial fisheries (SEDAR 2014).

The sex ratio for the Gulf Greater Amberjack stock is currently assumed to be 1:1 (SEDAR 2014). It is evident, however, that overall sex ratio estimates in the Gulf can vary from approximately 1:1 (nonlethal sexing) to 1:1.7 (Murie and Parkyn 2008 data set) to 1:2.5 (0.4 male to 1 female; Thompson et al. 1999). Factors that may lead to varying sex ratio estimates from the different studies include region-specific differences and fisheries-dependent versus fisheries-independent sampling. Nonlethal sampling allows the entire catch to be sampled, whereas sex ratio estimates based on Murie and Parkyn (2008) were obtained primarily (~80%) from the port sampling of legal-sized fish; hence, data on the sex of discarded fish were not available for a large proportion of that data set.

Sex ratios for 1,000-mm FL and larger Greater Amberjacks from both the Gulf (Beasley 1993; Thompson et al. 1999; current study) and the U.S. South Atlantic (Harris et al. 2007; Smith 2011) indicated that there was a relatively large female skew (~70% female) among these larger fish. This lends support to the speculation that the commercial Greater Amberjack fishery, which has a minimum length limit of 914 mm FL, has greater selectivity for female fish. Determining the sex of the landed commercial catch through the use of the urogenital features (or from collection of gonads) could confirm or refute this speculation. Female skewing among these larger individuals could arise from the faster growth rates that have been observed in Greater Amberjack females (Harris et al. 2007; Murie and Parkyn 2008) or could be attributable to some other factor, such as greater natural mortality of Greater Amberjack males, although the process by which this would occur is currently unknown.

In both the nonlethal sexing data set and the Murie and Parkyn (2008) data set used for the sex ratio analysis, there was some evidence for possible regional and temporal skewing of sex ratios. Highly skewed sex ratios have occurred in individual fishing areas and even within individual schools of Greater Amberjacks (Smith 2011). For instance, the sex ratio at one specific fishing location off the coast of northwestern Florida during March showed male skewing as high as 11:1, and this entire region had a male-skewed sex ratio of approximately 3:1 during the spring (Smith 2011). These data point to possible changes in sex ratios throughout the year within a particular spatial region (e.g., sex ratio changes based on migratory patterns or in relation to the spawning season). In the future, as tagged Greater Amberjacks that were sexed and released by Murie et al. (2013) are recaptured, the spatial and temporal distribution of sexes in this species may become evident. More comprehensive sampling of sex for the landed catch (i.e., representative samples from throughout the Gulf and throughout the year) would also be beneficial for elucidating patterns in the distribution of each sex.

The range of probable sex ratio values observed in this study based on nonlethal, fisheries-independent, and fisheries-dependent sampling should be considered in the future assessments for the Gulf Greater Amberjack stock. The most recent stock assessment (SEDAR 2014) considered the Gulf Greater Amberjack stock to be overfished and considered overfishing to still be occurring, despite stringent regulations for minimum size limits, bag limits, seasonal closures, and quotas. The disproportionate, female-skewed sex ratio for 1,000-mm FL and larger fish is of particular concern since...
females of these large sizes could contribute significantly to spawning stock biomass. Age-specific sex ratios over time are used in stock assessments of protogynous hermaphrodites, such as the Red Porgy (SEDAR 2012), due to their sex change and due to possible overexploitation of the male proportion of the population in particular. Similar stock assessment models that incorporate size- or age-specific sex ratios for the Greater Amberjack (i.e., fish < 1,000 mm FL and fish ≥ 1,000 mm FL) may be able to better capture the dynamics of the stock.

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