Abstract.—Restriction-fragment length polymorphism (RFLP) analysis of mitochondrial mtDNA was used to identify morphologically similar eggs of spring spawning sciaenids in lower Chesapeake Bay. During spring 1990 and 1991, ichthyoplankton surveys were conducted in lower Chesapeake Bay to estimate seasonal egg production and population biomass of black drum, *Pogonias cromis*. Rearing experiments indicated that at least three species of sciaenid (silver perch, *Bairdiella chrysoura*; weakfish, *Cynoscion regalis* and *P. cromis*) were spawning in the survey area during both years. Specific identification of eggs based on previously published ranges of outside egg diameter (OED) were not reliable because of considerable overlap in diameter distributions. However, analysis of weekly OED frequencies revealed the presence of three modes which differed in temporal occurrence, suggesting the products of three species. Genetic typing of eggs using RFLP analysis of mtDNA confirmed the presence of three species, but demonstrated that eggs of certain size classes represented two species. These results illustrate that reliance on previously published ranges of egg diameter for specific identification of spring-spawning sciaenids may overestimate the spawning biomass of black drum in Chesapeake Bay by as much as 50% owing to misidentification of weakfish eggs.

Morphometric and genetic identification of eggs of spring-spawning sciaenids in lower Chesapeake Bay*

Louis B. Daniel III
John E. Graves**

School of Marine Science
Virginia Institute of Marine Science
The College of William and Mary
Gloucester Point, Virginia 23062

At least five species of the family Sciaenidae (silver perch, *Bairdiella chrysoura*; spotted seatrout, *Cynoscion nebulosus*; weakfish, *C. regalis*; northern kingfish, *Menticirrhus saxatilis*; and black drum, *Pogonias cromis*) are purported to spawn during the spring in lower Chesapeake Bay (Joseph et al., 1964; Lippson and Moran, 1974; Johnson, 1978; Brown, 1981; Olney, 1983; Cowan et al., 1992). The eggs of spring-spawning sciaenids in lower Chesapeake Bay are morphologically similar, ranging in outside egg diameter (OED) from 0.66 to 1.18 mm, and having single or multiple oil globules of varying sizes (Johnson, 1978; Olney, 1983). As a result, specific identification of eggs based on morphological criteria is problematic. Holt et al. (1988) suggested that it may not be possible to determine the specific identity of sciaenid eggs from morphological criteria; Joseph et al. (1964) reported that positive identification could only be achieved with supplemental hatching studies.

Hatching studies have traditionally been used to identify morphologically similar eggs, including those of sciaenids. Joseph et al. (1964) cultured eggs of several sciaenids collected at a single station in southern Chesapeake Bay (16 May 1962) and raised larvae to an identifiable size (5–7 mm). The smallest eggs (0.630–0.777 mm) were found to be *B. chrysoura,* whereas the larger eggs (0.814–1.110 mm) developed into *P. cromis.* Culture of eggs (0.777–0.950 mm) collected during early June produced no *P. cromis* but did result in larvae of *B. chrysoura* and *C. regalis.* In contrast, Olney (1983) suggested that eggs of *P. cromis, C. regalis, B. chrysoura,* and *Menticirrhus* spp. were included in a size-frequency distribution of morphologically similar eggs collected from May through August in lower Chesapeake Bay, but that identifications based on diameter were ambiguous because of the high degree of overlap in diameter distributions (Table 1). Confounding this problem is the observation that egg size may change with varying salinity or as the spawning season progresses (Johnson, 1978).

Because many species of Sciaenidae in lower Chesapeake Bay spawn concurrently and have morphologically similar eggs, most studies have relied either on previously published egg size distributions or rearing for identification (Holt et al., 1985, 1988; Comyns et

*Contribution No. 1818 of the Virginia Institute of Marine Science.
**To whom reprint requests and correspondence should be sent.

Table 1
Reported range of outside egg diameter (OED) and study location for spring-spawning sciaenids.

Species	OED (mm)	Location	Author(s)
Bairdiella chrysoura	0.62–0.78	Chesapeake Bay	Joseph et al., 1964
	0.59–0.82	NW Gulf of Mexico	Holt et al., 1988
Cynoscion nebulosus	0.60–0.85	NW Gulf of Mexico	Holt et al., 1988
	0.70–0.85	NW Gulf of Mexico	Fable et al., 1978
Cynoscion regalis	0.68–1.18	Long Island Sound	Merriman and Sclar, 1952
	0.70–1.17	Chesapeake Bay	Pearson, 1929
	0.84–0.96	Delaware Bay	Wisner, 1965
Menticirrhus saxatilis	0.80–0.85	New Jersey	Welsh and Breeder, 1923
Menticirrhus spp.	0.63–0.87	NW Gulf of Mexico	Holt et al., 1988
Pogonias cromis	0.82–1.02	Chesapeake Bay	Joseph et al., 1964
	0.90–1.20	NW Gulf of Mexico	Holt et al., 1988

al., 1991; Saucier and Baltz, 1992; Saucier et al., 1992). However, the misidentifications that can result from overlapping egg-diameter distributions and the time-consuming nature of culture experiments make methods based on other characters desirable.

The application of biochemical genetics has provided an alternative to culture for positive identification of morphologically similar eggs. Electrophoresis of water-soluble proteins (allozyme analysis) has been used to distinguish between larvae and juveniles of morphologically similar species of marine fishes (eg. Morgan, 1975; Smith and Benson, 1980; Graves et al., 1988). Similarly, restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA has been employed to discriminate between the eggs of three congeneric serranids that could not be unambiguously identified with a single allozyme locus (Graves et al., 1990). More recently, direct sequencing and RFLP analysis of DNA amplified by the polymerase chain reaction (PCR) have been used to identify morphologically similar larvae of invertebrates (Olson et al., 1991; Silberman and Walsh, 1992).

In this paper we report that identification of eggs of sciaenids in lower Chesapeake Bay during spring based on published morphological criteria, rearing experiments, and genetic analysis are inconsistent. These results indicate that it is not possible to identify sciaenid eggs accurately by using diameter as the sole criteria. In addition, we present the results of weekly plots of egg size-frequency distributions and a RFLP analysis of mtDNA to determine the specific composition of eggs of sciaenids that may be present in lower Chesapeake Bay during spring.

Material and methods

Weekly zooplankton surveys of the lower Chesapeake Bay were conducted during April and May 1990 and 1991 to determine the distribution and abundance of eggs of black drum for an estimate of seasonal egg production. Samples of eggs were obtained with an *in situ* silhouette photography system consisting of paired 60-cm diameter, 335-μ nets fitted to a rigid frame (see Olney and Houde, 1993, for a detailed gear description). All deployments were 5-minute, stepped-oblique tows and yielded a standard plankton sample and a replicate film record. Plankton samples were preserved in 5–8% buffered formalin and sciaenid eggs were identified by using the criteria of Lippson and Moran (1974) and measured to the nearest 0.025 mm with a Zeiss Stemi SR stereomicroscope. Ten subsamples of eggs (n=75–100) sorted from preserved plankton samples were remeasured to assess measurement error.

During several cruises in May 1990 and 1991, eggs were collected in an area off the city of Cape Charles, Virginia, with a 0.5-m Hansen net fitted with 202-μ mesh to seed 1-liter Imhoff settling cones for hatching experiments. Eggs were originally separated as Type I (<0.80 mm) and Type II (>0.85 mm) based on the morphological criteria of Joseph et al. (1964). Rearing chambers were returned to the laboratory and held for 3 to 14 days. In these, larvae were periodically sac-

rificed and preserved in 5–8% buffered formalin. Identifications of preserved sciaenid larvae from pigment characters were based on Ditty (1989).

Sciaenid eggs collected in the same area during spring 1991, 1992, and 1993 were sorted from fresh plankton samples. To avoid contamination by the morphologically similar eggs of the cynoglossid *Symphurus plagiusa* and the soleid *Trinectes maculatus* that contain several oil globules and are abundant in lower Chesapeake Bay during the spring, all eggs with >3 oil globules were omitted from the samples. Although eggs of most spring-spawning sciaenids generally possess three or fewer oil globules (usually two) those of *Menticirrhus saxatilis* may contain from 1 to 16 oil globules (Johnson, 1978). After sorting, eggs were measured, placed in scintillation vials with 26 ppt seawater, and frozen at –70°C for genetic analysis. Individual eggs were thawed and remeasured prior to homogenization to assess shrinkage.

Sciaenid eggs were genetically typed by comparing mtDNA restriction fragment patterns of individual eggs with those of known adults. To obtain patterns of known adults, mature female sciaenids (*B.chrysoura, C. nebulosus, C. regalis, M. saxatilis,* and *P. cromis*) were collected by pound net, trawls, and hook and line in April and May 1990 and 1991. Ovarian tissue was excised and frozen at –70°C. MtDNA was purified from ovarian tissue by cesium chloride equilibrium density gradient ultracentrifugation following the protocols of Lansman et al. (1981). To determine a restriction enzyme that unambiguously identified the different sciaenid species, aliquots of mtDNA were individually digested with the following restriction enzymes: *Apa*I, *Ava*I, *Ban*I, *Ban*II, *Hind*III used according to manufacturer's instructions. The resulting fragments were separated electrophoretically on 1.0% agarose mini-gels run at 5 V/cm for four hours and visualized with ethidium bromide.

MtDNA-enriched genomic DNA was isolated from individual eggs following the protocols of Graves et al. (1990). Entire DNA samples were digested with a single discriminating restriction endonuclease, separated electrophoretically, and transferred to a nylon filter (Southern transfer) following standard protocols (Sambrook et al., 1989). Filters were hybridized with highly purified black drum mtDNA, nick-translated with biotin-7–dATP, washed, blocked and visualized following the methods of Graves et al. (1990).

Results

A total of 10,803 sciaenid eggs was sorted from samples collected in 1990 and 1991. Outside egg

diameter of all specimens ranged from 0.650 to 1.12 mm. Successive blind readings of samples of 75 to 100 eggs were used to assess measurement error. No differences were found in the size-frequency distributions indicating good agreement within the 0.025–mm size classes (two-sample t-test, $P<0.05$, $n=79$).

Qualitative analysis of culture experiments using the two egg types of Joseph et al. (1964) revealed the presence of three species. Cultures containing eggs designated Type I (<0.80 mm) resulted in larvae of *B. chrysoura*, whereas cultures of eggs designated Type II (>0.85 mm) resulted in larvae of *C. regalis* and *P. cromis*.

Analysis of preserved ichthyoplankton samples from 1990 and 1991 revealed the presence of larvae of *B. chrysoura, C. regalis,* and *P. cromis*. No early life history stages of other sciaenids were identified; however, yolk-sac larvae could not be identified to species. Because rearing studies and analysis of field-caught plankton samples revealed the presence of more than two species, we could not rely on the criteria of Joseph et al. (1964) for specific identification. We therefore examined weekly frequency of occurrence of all sciaenid eggs during 1990 (Fig. 1) and 1991 (Fig. 2). Based on temporal occurrence and size frequency we identified three modes. The largest eggs (>0.975 mm), Type C, were most abundant during the period 23 April through 9 May. Type-C eggs declined in abundance throughout May in both years. Mid-sized eggs (0.850–0.950 mm), designated Type B, generally appeared later than Types A and C. Type-B eggs did not exceed 5% of the total frequency of sciaenid eggs until 15 May 1990 and 9 May 1991. Type-B eggs increased in abundance from mid-May until the end of sampling. The smallest eggs (<0.850 mm), designated Type A, co-occurred with Type-C eggs; however, they did not exceed 5% of the total sciaenid eggs until 8 May 1990 and 9 May 1991. In 1990, Type-A eggs peaked in abundance on 15 May and gradually declined throughout the sampling period. In 1991, Type-A eggs were most abundant during the last sample on 28 May.

To test the hypothesis that eggs designated Types A, B, and C were separate species assemblages, the mtDNA restriction fragment patterns of known adult sciaenids were compared with those of fresh egg samples separated into Types A, B, and C. Restriction fragment length polymorphism analysis of mtDNA, purified from adult *B. chrysoura, C. nebulosus, C. regalis, Menticirrhus saxatilis,* and *P. cromis*, revealed species-specific restriction fragment patterns for each of the five enzymes. Of the five enzymes, *Hind*III showed the greatest differences between species, facilitating visualization with the Southern blotting procedure (Table 2).
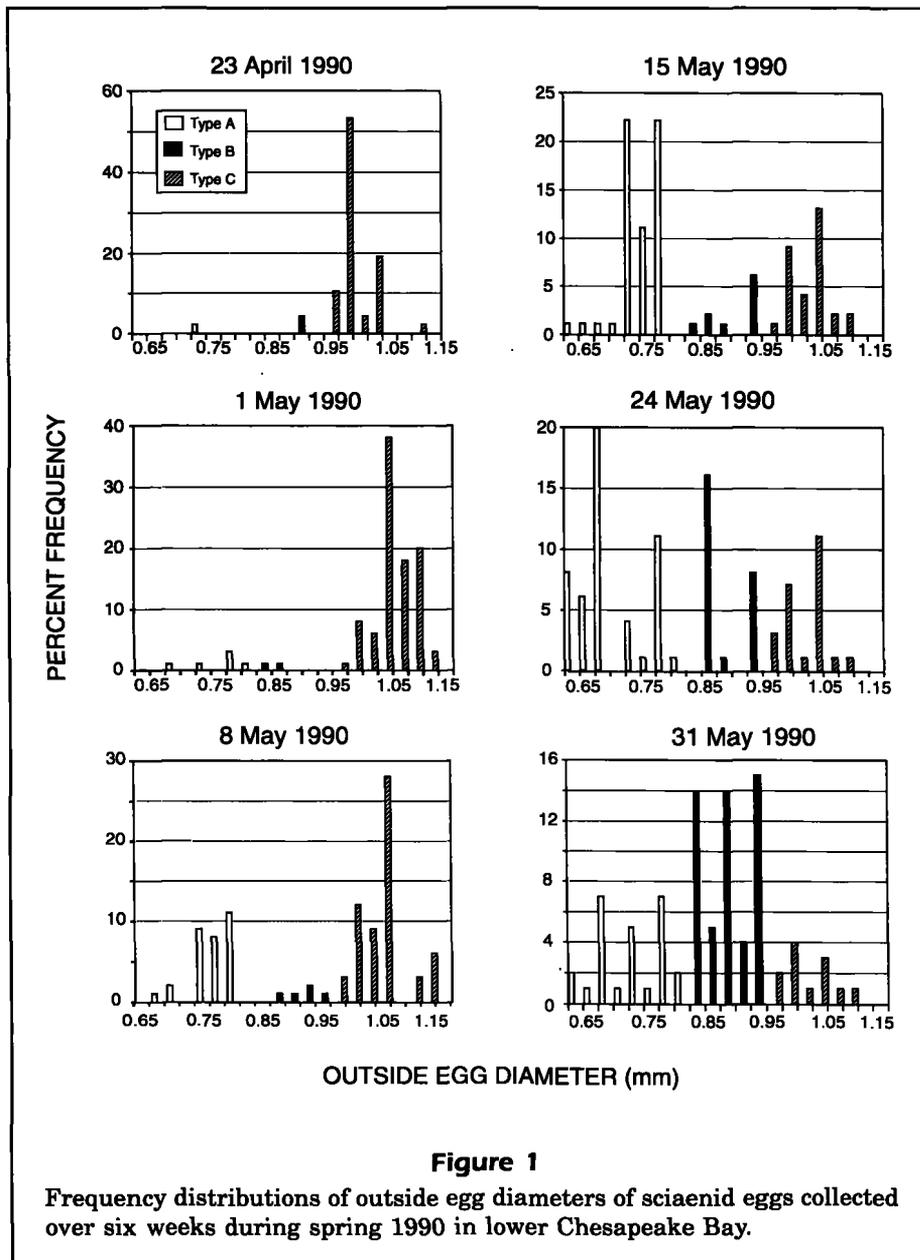
Figure 1

Frequency distributions of outside egg diameters of sciaenid eggs collected over six weeks during spring 1990 in lower Chesapeake Bay.

A total of 62 eggs, representing all sciaenid egg size classes collected in lower Chesapeake Bay, was identified with diagnostic *Hind*III restriction fragment patterns. *Bairdiella chrysoura*, *C. regalis*, and *P. cromis* were the only species of sciaenids identified; no other restriction fragment patterns were observed. Genetic identification of eggs designated Type A (<0.850 mm, *n*=12) resulted in 11 individuals of *B. chrysoura* and one specimen (0.825-mm OED size class) of *C. regalis* (Fig. 3). *Cynoscion regalis* composed the majority of type-B eggs (0.850-0.975 mm, *n*=18) analyzed, but seven of the 10 largest type-B eggs (0.975-mm OED size class) were identified as black drum. Type-C eggs, those 1.00

mm and larger (*n*=32), all possessed the restriction fragment pattern diagnostic for *P. cromis*.

Discussion

Identifications of eggs of sciaenids are often based on published diameter distributions or hatching experiments, or both. Results of hatching experiments and genetic analysis in this study indicate that samples of eggs of a single size class may represent the products of two or more species. For example, eggs designated Type I (<0.80 mm) and identified as silver perch by Joseph et al. (1964) were shown with genetic analysis to contain eggs of both weakfish and silver perch. Similarly, eggs designated Type II (>0.85 mm) and identified as black drum by Joseph et al. (1964) were shown with rearing and genetic analysis to contain eggs of both weakfish and black drum.

During the present study, neither hatching experiments nor genetic analysis identified eggs as black drum that were smaller than 0.975 mm OED. While temporally limited, the results of this study suggest that the range in size for eggs of black drum (0.975–1.125 mm) in lower Chesapeake Bay may be more restricted than those previously reported.

The ranges of egg diameter overlapped for silver perch and weakfish. Eggs genetically identified as silver perch ranged in size from 0.650 to 0.825 mm, in agreement with previously reported size ranges for silver perch in the northwestern Gulf of Mexico (0.59–0.82 mm, Holt et al., 1988) and Chesapeake Bay (0.625–0.775 mm, Joseph et al., 1964). Although Holt et al. (1988) identified eggs of silver perch as small as 0.590 mm, no sciaenid eggs smaller than 0.650 mm OED were collected in the present study. Sizes of eggs genetically identified as weakfish were found to range from 0.825 to 0.975 mm in diameter. These values are comparable with those reported by Wisner (1965, 0.84–0.96 mm) but are narrower than

the range (0.68–1.18 mm) given by Merriman and Sclar (1952) for Block Island Sound, New York. While the range in sizes for silver perch and weakfish reported in this study agree with past research, overlaps in these ranges preclude the sole use of egg size for identification.

Neither Joseph et al. (1964), Olney (1983), nor the present study identified eggs of *C. nebulosus* or *M. saxatilis* in samples collected in lower Chesapeake Bay. Fable et al. (1978) described laboratory-spawned eggs of *C. nebulosus* from a single female and reported a mean diameter of 0.77 mm (range 0.70–0.85 mm). Although based upon a limited sample size, Fable et al.'s data indicate that eggs of *C. nebulosus* could be confused with eggs of *B. chrysoura*; however, no eggs in our limited sample of this size range (n=12) were genetically identified as *C. nebulosus*. A possible explanation for the lack of eggs of *C. nebulosus* in the present study may be the tendency for adults to spawn in or around vegetated areas (Brown, 1981). The absence of eggs of *Menticirrhus* spp. in this genetic analysis may be explained by our exclusion of eggs with greater than three oil globules. Additionally, *Menticirrhus saxatilis* reportedly spawns off front beaches and possibly offshore (deSylva et al., 1962); consequently, circulation in the bay may prevent eggs of this species from entering the survey area or they may be transported to areas that were not sampled in our study.

The identification of species-specific restriction fragment patterns for spring-spawning sciaenids is based on the assumption that there is limited intraspecific variation of the diagnostic restriction fragment patterns. Recent studies of the population genetics of spotted seatrout, black drum, and weakfish (Graves et al., 1992; Gold et al., 1993) indicate that these species exhibit low intraspecific mtDNA variability. Furthermore, no variation of the *Hind*III fragment pattern was found in a survey of mtDNA
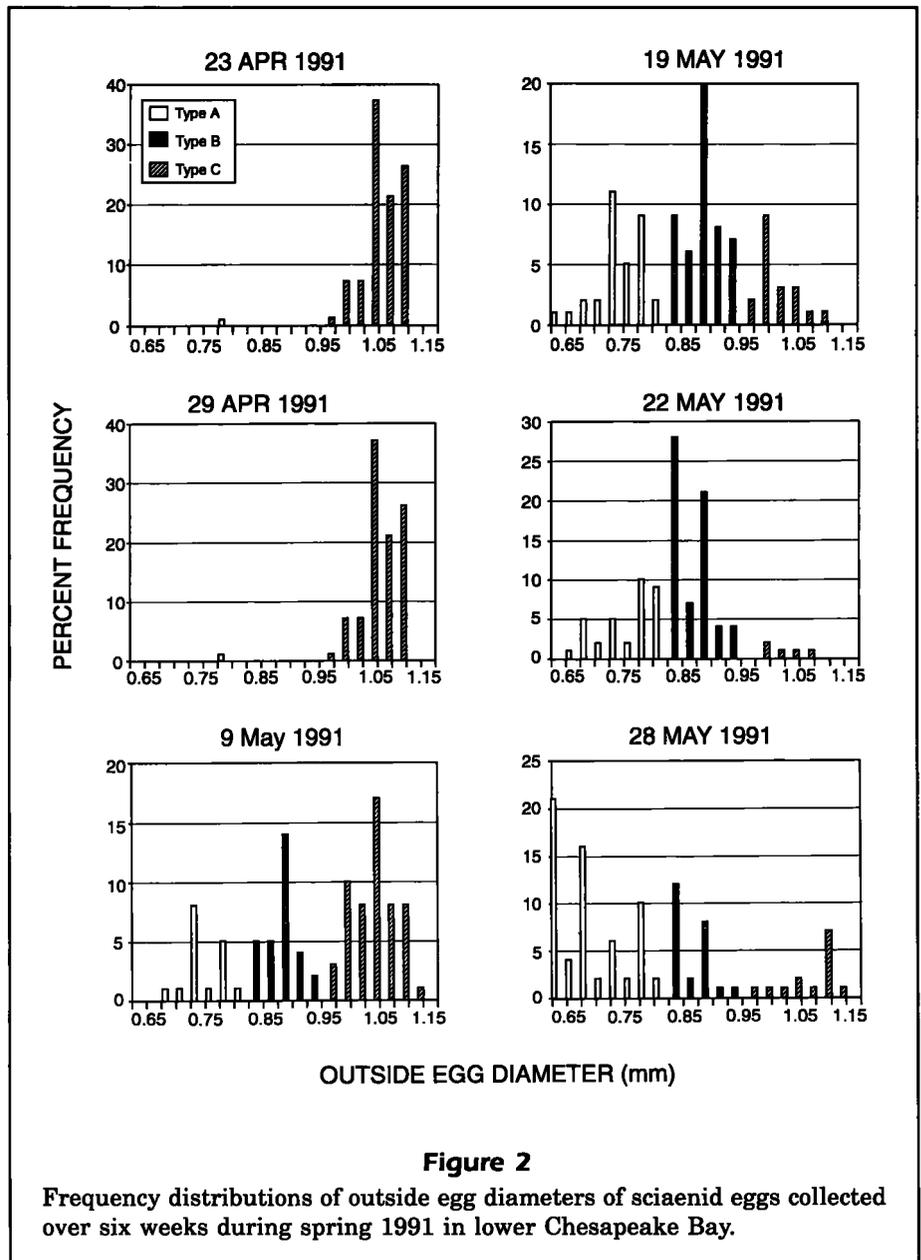

Figure 2

Frequency distributions of outside egg diameters of sciaenid eggs collected over six weeks during spring 1991 in lower Chesapeake Bay.

isolated from 25 adult *B. chrysoura* (L. Daniel, unpubl. data). Consequently, the common restriction fragment patterns used to distinguish species in this study were deemed suitable for use in identifications.

Variability in egg-size distributions with changing salinity and over the spawning season were not examined in this study. Consequently, exact size groupings may only be applicable to the particular salinity regime (19–25 ppt) that we sampled. However, samples were taken throughout peak spawning for black drum and silver perch and may encompass the ranges that occur for these species in lower Chesapeake Bay.

Results of our genetic analysis suggest that identifications of eggs of spring-spawning Sciaenidae in
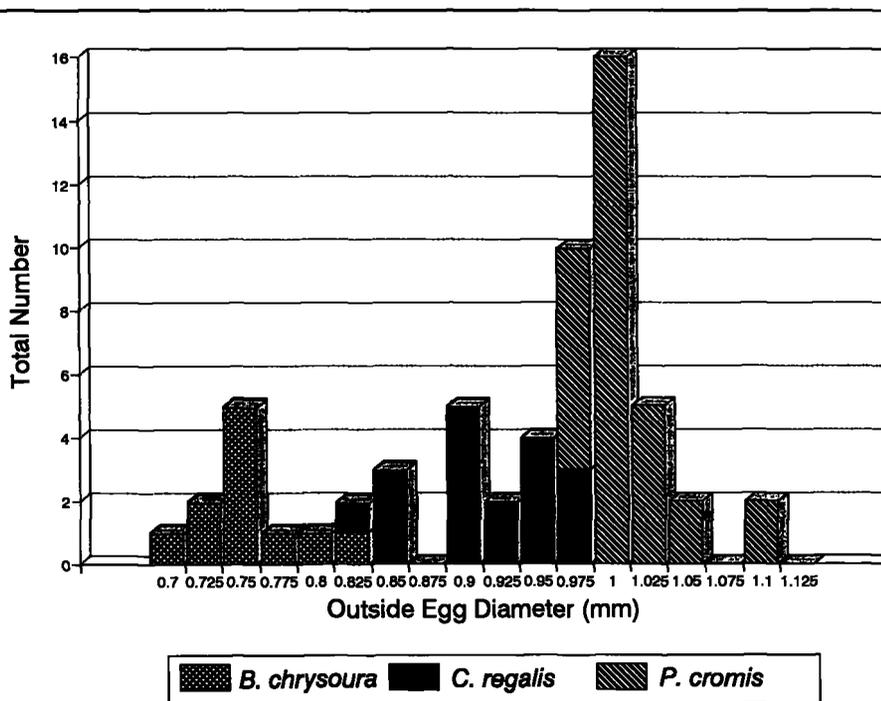
Table 2

Common fragment sizes produced by restriction endonuclease (HindIII) digestion of mtDNA purified from ovarian tissue of spring spawning sciaenids.

Species	Fragment sizes (Kb)						
Bairdiella chrysoura	5.0	3.9	2.8	1.9	1.7	1.7	1.3
Cynoscion nebulosus	8.5	4.5	3.8[1]				
Cynoscion regalis	5.6	4.3	4.1	2.9			
Menticirrhus saxatilis	5.4	3.2	2.4	2.0	1.9	1.8	
Pogonias cromis	3.3	2.9	2.7	2.5	2.1	1.3	1.0

[1] J. Gold, Texas A&M, College Station, TX, pers. commun. 1993.

Figure 3

Size distributions of all eggs morphologically typed as sciaenids and identified using genetic techniques.

wise, measures of spawning stock biomass will be similarly over-estimated, results that could significantly impact management decisions. Comparable biases in estimates of egg production and spawning stock biomass of weakfish could result from egg misidentifications. However, the more protracted spawning season and greater area of spawning for weakfish in Chesapeake Bay (Olney, 1983) would make these impacts much less severe.

Biochemical techniques are an important tool for the further study of eggs of sciaenids. Genetic analysis has the potential to produce reliable results and permit the storage of samples for later analysis. Additional studies are needed to survey genetic identifications over the entire spawning season and area to determine if egg sizes change over time or are influenced by seasonal changes in hydrography or by age structure of the spawning stock. Finally, the use of genetic techniques, coupled with an extensive examination of morphology could lead to the delineation of other characters that may be useful in separating the eggs of these species.

Acknowledgments

lower Chesapeake Bay based on OED are subject to error. These findings are particularly timely in light of the increased use of fishery-independent assessments of stock size that require precise estimates of egg abundance (egg production method). Because eggs of black drum and weakfish are spatio-temporally coincident and OEDs overlap, estimates of egg production by black drum in lower Chesapeake Bay may be over-estimated by 50% or greater if identification criteria are based solely on egg size. Like-

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