Defining a spawning population (spotted seatrout *Cynoscion nebulosus*) over temporal, spatial, and demographic scales

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ABSTRACT: Fish spawning populations are complex and affected by many factors acting over temporal, spatial, and demographic scales. To better understand these factors, we chose to study spotted seatrout *Cynoscion nebulosus*, which has closed populations over small spatial scales and a periodic life-history pattern. We used experimental gill nets and hook and line, within a spatially and temporally explicit sampling design, to collect 2034 spotted seatrout in lower Tampa Bay, Florida, USA, in 2001 and 2002. Ovarian development was evaluated histologically. Mature females did not all spawn throughout the spawning season, and first-time spawners (Age 1) had the lowest probability of doing so. Two reproductive behaviors were observed: fish moving in the late afternoon or evening to a high-intensity spawning site in an inlet and low-intensity spawning within the estuary. Larger, older female and male spawners were more abundant at the inlet site than at nearby estuarine sites. Both batch fecundity and the percentage of females spawning increased with age and size. Spatial–temporal interactions made it difficult to assess certain aspects of reproduction, such as spawning frequency.

KEY WORDS: Fish spawning · Spawning population · Spatio-temporal · Demographics · Reproduction

INTRODUCTION

Conserving the spawning population of a stock is a fundamental goal of marine fisheries management. Given that approximately 25 % of the world's fisheries are considered overexploited and that breeding populations of many stocks have been drastically reduced (Hutchings & Reynolds 2004), there is a realization that traditional management has often failed to meet this goal (Frank & Brickman 2001). Fisheries-management reference points for many stocks are based on spawning-stock biomass (SSB) and its relationship to fishing mortality, either directly by using spawning potential ratios or indirectly by using the steepness of the spawner–

recruit relationship. Both methods are based on 2 underlying assumptions: (1) that egg production can be predicted based on SSB and (2) that egg production drives reproductive success.

As our understanding of the reproductive dynamics of marine fishes improves, it is becoming increasingly clear that these assumptions may be too simplistic. The assumption that egg production can be predicted based on SSB is violated if SSB does not properly reflect the spawning population, such as when skipped spawning occurs (Rideout et al. 2005, Secor 2008) or if aspects of the spawning population other than biomass (e.g. demographics, condition) significantly affect egg production (Murawski et al. 2001, Marshall et al. 2003,

Berkeley et al. 2004). In addition, the assumption that the number of eggs produced drives reproductive success is being increasingly questioned. The reproductively successful population in any given year may be considerably smaller than the adult population and be driven by temporal, spatial, and demographic factors (Hedgecock 1994, Berkeley et al. 2004, Hutchings & Reynolds 2004).

Although the need to better define spawning populations over temporal, spatial, and demographic scales has been recognized (Rowe & Hutchings 2003), meeting this need is difficult. To optimize reproductive success, fish have developed a wide range of reproductive strategies made up of physiological, environmentally driven, and behavioral traits (Murua & Saborido-Rey 2003, Lowerre-Barbieri 2009). Thus, given the complexity of reproductive dynamics, the wide geographic range of many marine stocks and the lack of established methodology to determine spawning sites (Lawson & Rose 2000), it is often difficult to conduct high-resolution research on spawning populations.

Spotted seatrout *Cynoscion nebulosus* is an ideal species for reproductive-dynamics research because of its population structure and its life-history pattern, which are similar to those of other exploited species. Spotted seatrout in the Gulf of Mexico (GOM) show little geographic movement (Hendon et al. 2002). The population structure is believed to be a series of overlapping subpopulations, each centered in a natal estuary, with relatively little mixing between them (Jones 2006). Spotted seatrout are highly fecund, multiple-batch spawners that produce small, pelagic eggs (Brown-Peterson 2003). They demonstrate a periodic life-history strategy, as do most recreationally and commercially targeted species (Winemiller 2005). They have moderate to low longevity (maximum reported age of 12 yr; Maceina et al. 1987) and relatively short generation times (most fish mature by Age 1; Brown-Peterson 2003). Spotted seatrout are the most targeted sport fish in the GOM (NMFS 2007), and GOM subpopulations exhibit age truncation (maximum observed ages are from 5 to 9 yr; Nieland et al. 2002).

In the present study, we evaluated a number of hypotheses about spawning populations and how they are distributed over temporal, spatial, and demographic scales. Temporally, we determined spawning seasonality and tested the hypothesis that all mature females spawn throughout this time period. Spatially, we tested the hypothesis that spawning activity was equally distributed throughout the study area. Temporal–spatial interactions were tested to evaluate if fish movement is associated with spawning. The demographics of the spawning population were assessed over space and time to test whether age or size affected the probability of spawning, spawning site selection, or batch fecundity. Our results are discussed in the context of a companion study that mapped spotted seatrout spawning sites throughout Tampa Bay (Walters et al. 2009).

MATERIALS AND METHODS

Collections of samples. Sampling was conducted in lower Tampa Bay, Florida, USA (from approximately 27° 27' N to 27° 44' N) in 2001 and 2002. The lower bay was chosen because it was reported to have the highest level of spotted seatrout *Cynoscion nebulosus* spawning within the bay (McMichael & Peters 1989). Four sampling zones, and sites within them, were chosen based on reported spotted seatrout spawning habitat: the presence of different seagrasses (turtle grass *Thalassia testudinum*; manatee grass *Syringodium filiforme*; or shoal grass *Halodule wrightii*) bordered by slightly deeper water (Brown-Peterson 2003). However, in August 2001, a spawning site was discovered in an inlet between 2 barrier islands and added as a fifth sampling zone (Fig. 1).

Due to the discovery of this spawning site, the sampling design differed between years. In 2001, we sampled twice weekly from January to mid-October. Estuarine sampling zones were chosen randomly, and 2 or 3 sites per zone were sampled per trip. Because spotted seatrout spawn at dusk (Brown-Peterson 2003), sampling alternated between morning (AM) and afternoon/evening (PM) to ensure equal sampling at times associated with spawning, when fish may aggregate and spawning females will not be randomly distributed (PM) as well as times not associated with spawning (AM). The spawning site at the inlet was discovered on 6 August 2001, after receiving a tip from an angler. This site was bordered by Zone 1, but occurred outside the expected spawning habitat (Fig. 1). The inlet site was designated as both a site (given its small size) and as a zone (Zone 5), to distinguish it from estuarine sampling. Preliminary sampling of the inlet site was conducted approximately weekly from August through September 2001. By 8 October 2001, spawning had ended and winter sampling was decreased to PM trips. One estuarine zone was sampled per week, and the inlet was sampled every other week.

In 2002, sampling was modified to focus on the inlet and the 2 adjacent estuarine zones (1 and 2). Winter sampling continued through March, but data from Zones 3 and 4 were removed from the analysis to be consistent with the remainder of the year's sampling. Beginning in April, Zones 1 and 2 were sampled in the AM and PM approximately monthly through September. The inlet site was sampled weekly in the PM and approximately monthly in the AM (few fish were
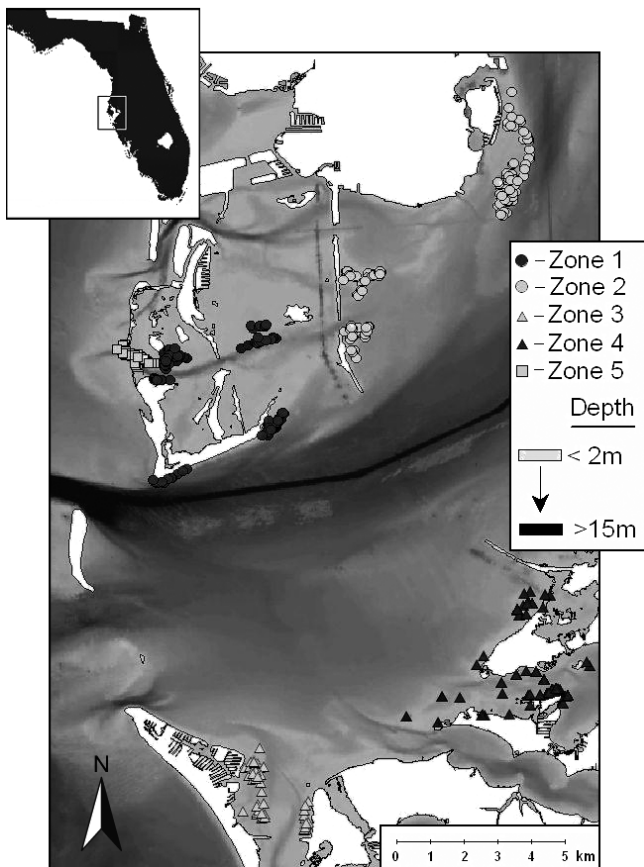
Fig. 1. Sampling sites and zones in lower Tampa Bay, Florida, USA. Each symbol represents a sampling event. Tampa Bay's location in Florida is indicated in the inset

caught in the AM in preliminary sampling) from March through September. Sampling effort was again decreased to PM trips beginning in October.

Sample gear included an experimental gill net (GN) and hook and line (HL). The primary sampling gear was a 3.05 m deep, 228.6 m long, monofilament (104 twine size), multipanel experimental GN. Panels were 45.7 m long and made of the following stretched mesh sizes: 6.35, 7.62, 8.89, 10.16, and 11.43 cm. Duration of fishing (from release of net to begin of haul-in) was 20 min. Three anglers fished on any given date, and the duration of their HL fishing was recorded. Each unique combination of sampling gear and site per date was considered a sampling event. Time and location of each event were recorded. A Garmin 235 GPS/sound with a Garmin GBR 21 DGPS beacon receiver was used to record location.

Fish were kept on ice until they were processed in the laboratory, where they were measured for total length (TL, ±1 mm), total weight (TW, ±1 g), gonad weight (GW, ±1 g), and stomach weight (STW, ±1 g). Somatic weight (SW) was estimated as TW – (GW + STW). Sex was determined, gonad development

assessed, and both sagital otoliths removed and stored dry. Within the spawning season, males were classified as sexually immature, mature, or in spawning condition (excised testes expelled milt on pressure). Ovaries were macroscopically examined for the presence of yolked oocytes and hydrated oocytes. For histological analysis, ovarian tissue was fixed in 10% neutrally buffered formalin for 24 h, soaked in water for 24 h, and stored in 70% ethanol. Samples were embedded in paraffin, sectioned to 5–6 μm thickness, and stained with Hematoxylin and Eosin Y.

Ovarian analysis. Ovaries were examined histologically. Four oocyte developmental stages were identified: primary growth, cortical alveoli, yolked, and maturation. The following characteristics of oocyte maturation (OM) were noted: germinal vesicle migration (GVM), germinal vesicle breakdown (GVBD), yolk coalescence or clarification, and oocyte hydration (Jalabert 2005). Postovulatory follicles (POFs) were classified as either newly collapsed (recognizable by the size and appearance of the granulosa cells' nuclei), 12 h, 24 h, or 36 h. POF age was based on POF size, organization, and elapsed time from peak spawning (Hunter & Macewicz 1985).

Histological indicators of spawning differ in their duration times, affecting how they can be used in reproductive analyses. Imminent spawning was based on the early stages of OM, which in spotted seatrout, can precede spawning by 6 to 14 h (Brown-Peterson 2003) and can only be identified histologically. Early OM included GVM, but little yolk coalescence or hydration (Fig. 2A) and signified the reception of the appropriate gonadotropic signal to undertake final meiosis (Jalabert 2005). Active spawning was designated by 3 histological indicators: late OM, ovulation, and newly collapsed POFs (Fig. 2B–D). Late OM was characterized by completed GVM or GVBD, yolk coalescence, and sufficient hydration that hydrated oocytes were detectable macroscopically. Ovulation indicated females in the process of actively spawning. Ovulation and newly collapsed POFs are extremely short-lived histological indicators and were used as the most conservative way to assess spawning time and location. Recent spawning was indicated by degenerating POFs (12 to 36 h old).

The above histological indicators were then used to develop a tiered ovarian classification system (Table 1) that allowed us to evaluate reproduction over different temporal scales: lifetime, annual, intraseasonal (i.e. spawning frequency), and diel. The lifetime scale was defined by whether a fish was mature or not. The annual reproductive scale included 6 reproductive phases: immature, developing, spawning-capable, spawning, regressing, and regenerating (Brown-Peterson et al. 2007). The spawning-capable phase indicated females
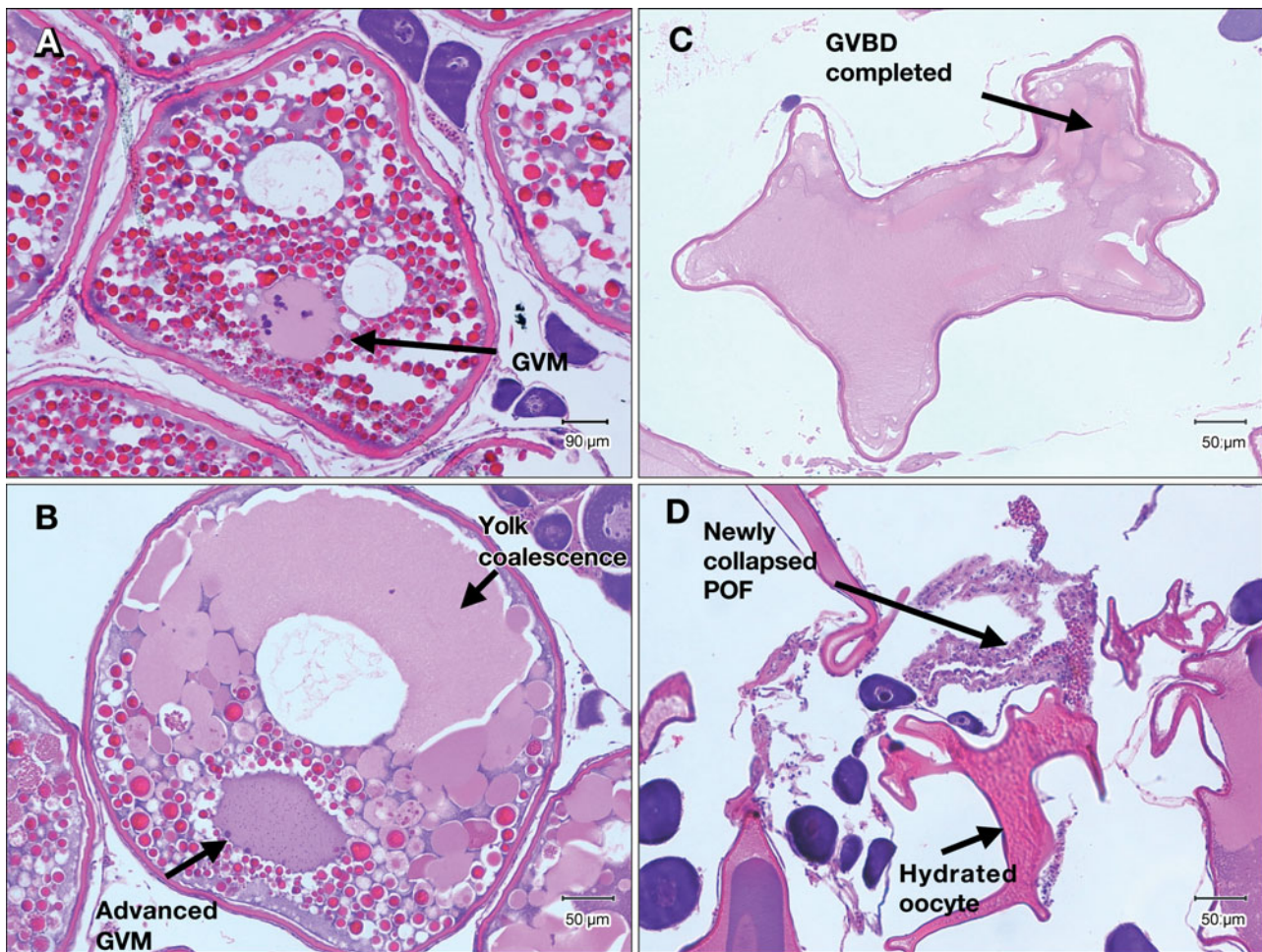
Fig. 2. *Cynoscion nebulosus*. Histological indicators in spotted seatrout: (A) early oocyte maturation (germinal vesicle migration [GVM]), (B) late oocyte maturation (GVM and yolk coalescence), (C) late oocyte maturation (germinal vesicle breakdown [GVBD] and hydration), and (D) a newly collapsed postovulatory follicle (POF)

sufficiently developed (i.e. with fully yolked oocytes) to spawn after receiving the appropriate endocrine signal. They could either be recruiting to the spawning population for the first time or represent females in between spawning events. Spawning/spawners were defined as females with any histological indicator of spawning. These phases were further grouped to indicate reproductive state: (1) non-spawning, (2) spawning-capable, or (3) spawning. The reproductive states of spawning-capable and spawning were used to define the spawning population. Diel and intraseasonal cycles were assessed using the 3 subphases of spawning: imminent, active, or recent. Active spawning/spawners were defined as females within 2 h of spawning.

Batch fecundity was estimated gravimetrically using the hydrated oocyte method (Hunter et al. 1985). Fresh oocytes were hydraulically separated from each other and from the ovarian membrane and preserved in 2% neutrally buffered formalin using the method of Lowerre-Barbieri & Barbieri (1993). Samples were stirred to reduce bias due to settling before removing 2 subsamples (≈0.1 g) and counting the number of hydrated oocytes. Estimates of batch fecundity were not used if histological analysis indicated ovulation had begun.

Otolith analysis. Fish were aged using transverse sections of otoliths (Maceina et al. 1987). Otoliths were sectioned through the core using a Buehler low-speed Isomet saw. Sections from 350 to 500 µm thick were mounted on glass slides with histomount. The number of annuli and the distance from the edge to the most recent annulus were determined by viewing the sections through a dissecting microscope, using transmitted light. Sections that were difficult to read were also viewed in reflected light. All slides were considered twice by 2 different readers. Ages were assigned to year classes by using a 1 January birth date (Jearld 1983) to clearly separate fish born in different years.

Table 1. *Cynoscion nebulosus*. Ovarian classification and terms based on histological analysis. POF: postovulatory follicle; OM: oocyte maturation; GVM: germinal vesicle migration; GVBD: germinal vesicle breakdown

Reproductive state	Phase	Histological indicators	Significance
Immature			
Non-spawning	Immature	Only oogonia and primary growth oocytes, including chromatin nucleolar and perinucleolar oocytes. Usually no atresia.	Virgin that has not yet recruited to the spawning population
Mature			
Non-spawning	Developing	Cortical alveolar and sometimes early yolked oocytes. No evidence of POFs. Some atresia may be present.	Environmental signals have triggered development, but fish are not yet developed enough to spawn
Mature spawning population			
Spawning-capable	Spawning-capable	Yolked oocytes. May be some atresia.	Fish developed enough to spawn
Spawning	Spawning	OM, ovulation, or POFs.	Fish with indicators of spawning activity
	Subphases:		
	Imminent	Early OM (GVM with little yolk coalescence)	Will spawn in 14 h.
	Active	1. Late OM (completed GVM or GVBD with yolk coalescence and partial to full hydration) 2. Ovulation 3. Newly collapsed POFs	Spawning ±2 h
	Recent	POFs (12–36 h old)	Spawned within 2 d
Mature			
Non-spawning	Regressing	A high percentage of yolked oocytes undergoing atresia (alpha and beta)	Cessation of spawning
Non-spawning	Regenerating	Only primary growth oocytes present, including chromatin nucleolar and perinucleolar. Muscle bundles, enlarged blood vessels, thick and/or convoluted ovarian wall, and gamma or delta atresia may be present.	Sexually mature, reproductively inactive. Most common outside of the spawning season.

Data analysis. Before we could evaluate temporal, spatial, and demographic effects, we needed to assess whether sex or gear type affected the size of the fish sampled. Generalized linear models (GLMs) were used to examine these main effects and their possible interactions (PROC GLM in SAS). Because gear type significantly affected size (see 'Results'), it was accounted for in subsequent analyses.

The 4 reproductive temporal scales were used to select data at the appropriate scale for each hypothesis tested. This was a 2-step process, because the temporal scales first had to be determined. The population spawning season was defined based on the first and last occurrence of spawning females for each year. To assess diel periodicity, histological indicators associated with spawning activity (early OM, late OM, ovulation, and POFs) were evaluated by their time of occurrence. Based on spawning seasonality and diel periodicity, we categorized our data as collected during times when spawning was possible (i.e. within the spawning season and PM) or not. A categorical vari-

able, event type, was used to distinguish these 2 types of data (spawn time versus non-spawn time).

To test the hypothesis that all mature females spawn throughout the observed population spawning season, we assessed the percentage of females in the spawning population and their size by month (i.e. if recruitment to the spawning population was synchronous and the same population spawned throughout the season, the percentage of females in the spawning population should remain constant and the mean TL should not decrease). We calculated the percentage of females in each reproductive phase collected during the spawning season and considered the spawning-capable and spawning phases indicative of the spawning population. The monthly mean TLs of females in the spawning population were calculated as the least-squared means from a GLM after adjusting for gear (PROC GLM in SAS).

To test the hypothesis that spawning activity was equally distributed throughout the study area, we evaluated: (1) abundance by zone and its relationship to time of spawning and (2) the percentage of active

spawners by zone and event. To evaluate abundance patterns, the number of fish per event (e.g. catch per unit effort, CPUE) were modeled using GLMs (PROC GENMOD in SAS) with the following potential explanatory variables: zone (4 estuarine and 1 inlet), event type (spawn or non-spawn), gear (HL or GN), duration of fishing, and the interaction between zone and event type. Because catch data were skewed with a predominance of zero observations, we used a negative binomial distribution with a log-link function. A reduced model was then developed without event type as a main effect because it was not significant. A χ^2 test was used to analyze the proportion of active spawners in spawn-time events by zone. To ensure that estuarine spawning sites were not overlooked in the analysis by zone, the percentage of active spawners in each spawn-time sampling event (n $\geq$ 3) was calculated and compared to the overall percentage of active spawners at the inlet.

Temporal–spatial interactions were tested to evaluate migration into or out of the sampling area associated with spawning. We calculated the percentage of spawners by zone and time category (AM versus PM). The underlying assumption being tested was that spawners were not moving in or out of these zones and, thus, the percentage of spawners would be similar in both time categories. We modeled the proportion of estuarine spawners using GLMs (PROC GENMOD in SAS) with a binomial distribution and a logit-link function. Only mature females sampled in estuarine zones during the spawning season were analyzed. Females that were not spawning were categorized as 0, and spawning females were categorized as 1. Explanatory variables included gear, zone, and time category.

Demographics were assessed over 2 time scales, lifetime and annual. Size at 50% maturity was estimated separately for males and females collected in the period from March to May, using the following logistic equation:

$$P = \frac{1}{1+e^{[-a(L-L_{50})]}}$$

where P is the proportion mature at length L, a is the slope of the curve, and L_{50} is the length at 50% sexual maturity. The months from March to May were chosen, after evaluating spawning seasonality. These months had the fewest regenerating females, which are difficult to distinguish from immature females (Hunter & Macewicz 2003). Because fish mature only once in a lifetime, it was possible to identify first-time spawners by selecting developing females in the first age class with mature fish. All other demographic analyses were based on mature females within the spawning season.

The demographics of the spawning population were assessed over annual and interannual temporal scales

to test whether age or size affected the probability of being in the spawning population or of spawning. The proportion of females in the spawning population was modeled using GLMs (PROC GENMOD in SAS), a binomial distribution, and a logit-link function. A higher probability of being in the spawning population at time of capture was assumed to be associated with a longer spawning season for that age or size class. Females not in the spawning population were categorized as 0, and those in the spawning population were categorized as 1. Two models were developed with the explanatory variables of gear, zone, month, and either size or age. Size was grouped in classes of 50 mm TL. To have sufficient sample sizes in each category, only females in the 250 to 500 mm TL size range, Ages 1 to 5, and Zones 1, 2, and 5 were used. Gear was not significant for either model, and month was not significant for the age model, so these variables were left out in reduced models. To evaluate demographic effects on the probability of spawning, which should be analogous to the number of breeding opportunities in a year (i.e. length of individual spawning seasons and spawning frequencies), the proportion of spawning phase females in the spawning population was modeled using the same procedure. Spawning-capable females were categorized as 0, and spawning females were categorized as 1.

To evaluate if demographics were driving spawning site selection, we compared the size and age of the spawning population in the inlet with nearby estuarine Zones 1 and 2. To assess if there was a threshold size or age at which fish moved to the inlet to spawn, the size and age ranges of active spawning-phase females and spawning condition males were determined. To evaluate if the demographics of spawners at the inlet differed from those in nearby estuarine zones (Zones 1 and 2), mean TL and age of spawning population females and spawning condition males were calculated as the least-squared means from a GLM after adjusting for gear (PROC GLM in SAS).

Demographic effects on egg production were evaluated by combining the above analysis on the probability of spawning with an analysis of the effects of size and age on batch fecundity. The probability of spawning rather than spawning frequency was evaluated, because traditional methods to assess spawning frequency could not be used. Spawning frequency is typically estimated as the reciprocal of the percentage of females with either hydrated oocytes or POFs (Hunter & Macewicz 1985) and is based on the assumption that no net movement into or out of the sampling site occurs. Spatial analysis indicated this assumption was violated. Linear regression was used to model the basic relationship between batch fecundity (BF) and 2 measures of size (TL and SW). To linearize the parameters

in the BF to SW relationship, which was a power function, BF and SW were $\log_e$-transformed. To assess whether zone or age significantly affected BF, stepwise linear regression was used to model the relationship between BF and the following main effects: SW, zone, and age. The significance level for variable inclusion was 0.5. All statistical analyses were performed using SAS Version 9.13 (SAS Institute) and an alpha level of 0.05.

RESULTS

Sampled population

A total of 2034 spotted seatrout *Cynoscion nebulosus* were collected: 1189 in 2001 and 845 in 2002. Seven fish could not be aged because of broken otoliths, and 2 fish could not be sexed due to extremely undeveloped gonadal tissue and our inability to macroscopically distinguish it from digestive tissue. Fish ranged in size from 193 to 640 mm TL (Fig. 3), with an average size of 354 mm TL. Females, on average, were larger than males, with a female mean TL of 379 mm (n = 1118) compared to 324 mm TL for males (n = 905). All fish >535 mm TL were female (n = 51). Ages ranged from 0 to 8 yr for both sexes, and most fish (98%) were Age 5 or younger. The average age was similar for both sexes (females: 2.5 yr; males: 2.4 yr). Size differed significantly by sex (2-way ANOVA, $F_{1,2021} = 289.4$, p < 0.0001; Fig. 3) and collection gear (2-way ANOVA, $F_{1,2021} = 695.1$, p < 0.0001), with larger fish of both sexes collected by GN. There was also a significant interaction between gear and sex ($F_{1,2021} = 65.0$, p < 0.0001) because HL selected for smaller fish and females grow larger than males.

Temporal spawning activity

Spawning seasonality was asynchronous. Population spawning seasons were similar in 2001 and 2002. Spawning-capable and spawning females were collected from March through September (Fig. 4A): from 7 March to 19 September 2001 (193 d) and from 21 March to 23 September 2002 (195 d). These dates were used to define annual population spawning seasons. Within these seasons, 77% of sampled females were part of the spawning population, but percentages differed by month. From April through August, more than 75% of sampled females were part of the

spawning population (Fig. 4b), with greater than 85% occurring in April and May. These patterns suggest individual variability in spawning seasonality. Mean TL of spawning population females differed significantly by month ($F_{6,502} = 4.07$, p = 0.0005) and collection gear ($F_{1,502} = 66.46$, p < 0.0001). There was no significant interaction between main effects ($F_{6,502} = 1.70$, p = 0.12). The lowest mean monthly TL occurred mid-spawning season (Fig. 4B), indicating a relationship between fish size and spawning seasonality.

Active spawning occurred only in PM samples. Females with late OM were collected from 15:58 to 21:23 h, and ovulatory females were collected from 17:32 to 21:23 h. We assumed that fish with late OM were within about 2 h of ovulation based on the time lag between when these stages were first observed (arrow in Fig. 5). Early OM could not be used as a predictor of imminent spawning because it occurred in both AM and PM samples.
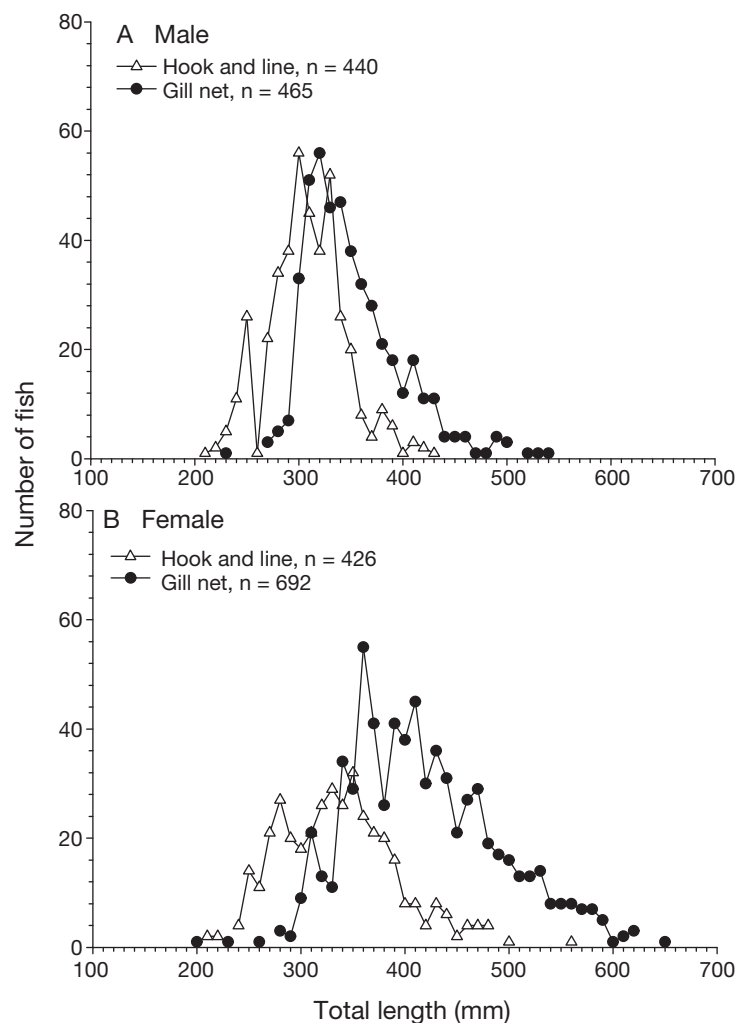

Fig. 3. *Cynoscion nebulosus*. Size distributions of (A) males and (B) females by collection gear
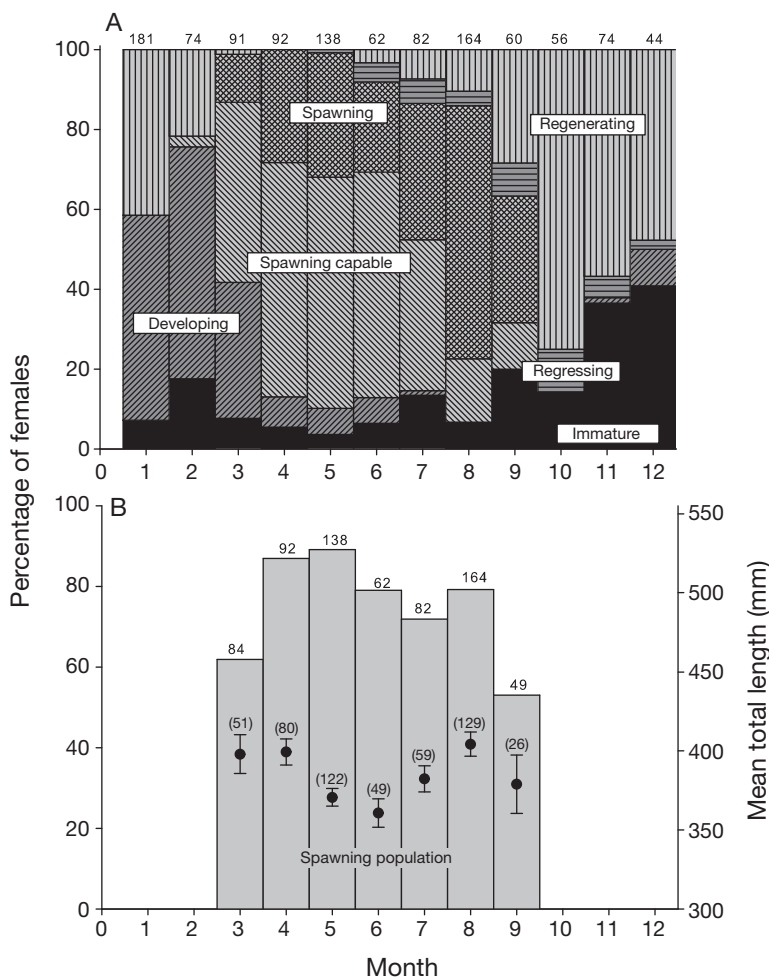
Fig. 4. *Cynoscion nebulosus*. (A) Monthly percentage (2001 and 2002 combined) of females by reproductive phase: immature, developing, spawning-capable, spawning, regressing, and regenerating and (B) percentage of females collected during the spawning season that were part of the spawning population (bars) and female spawning population mean total length (TL) ± 1 standard error by month (●). Means were calculated as the least-squared means from a general linear model after adjusting for gear. Sample sizes are indicated above bars (all females) or in parentheses (spawning population females)

Spatial spawning activity

There were spatial differences in abundance associated with time of spawning, indicating movement into and out of sampling zones. Mean predicted CPUE was greater in estuarine zones than in the inlet (Fig. 6A). However, CPUE in estuarine zones were lower in spawn-time events than in non-spawn-time events, and an opposite trend was seen at the inlet (Fig. 6B). The mean predicted CPUE in spawn-time events at the inlet (Zone 5) was 2.62 fish (89 events), and it was negligible at non-spawn times (0.43 fish, 65 events). Fish captured in the inlet during non-spawn-time events

were all male (n = 8 in the AM during the spawning season; n = 20 ± 2 to 3 wk of the spawning season). Because estuarine zones showed the opposite abundance pattern to that of the inlet zone, event type was not a significant main effect in the full model (negative binomial regression, n = 626, p = 0.70), but interactions between event type and zone were highly significant in the reduced model (negative binomial regression, n = 626, p < 0.001).

Spawning activity was not equally distributed throughout the study area. Two reproductive behaviors were observed: fish moving nightly to a high-intensity spawning site (the inlet) and low-intensity spawning within estuarine zones. The percentage of active spawning phase females in spawn-time events differed significantly by zone (χ^2 = 199.3, n = 319, p < 0.0001). Of the females from spawn-time events at the inlet, 91 % (n = 170) were in the active spawning phase. In contrast, the percentage of females in the active spawning phase in estuarine zones ranged from 0 to 16 % (Fig. 7). The percentage of hydrated females (active spawning and imminent spawning) at Zone 5 (the inlet) was 97 %. In comparison, the 2 estuarine zones with the highest percentages (Fig. 7) were Zone 1 (26 %) and Zone 2 (14 %). Estuarine zones had no spawn-time events with ≥90 % active spawning phase females (n ≥ 3 females event^{-1}), with the exception of 1 event just east of the inlet in Zone 1. Similarly, virtually all ovulatory females were captured in Zone 5 (n = 21), with the exception of 2 ovulatory females from just east of the inlet.

Because reproductive behavior led to fish moving in and out of our sampling zones, we could not estimate spawning frequency. Using traditional methods, the 97 % hydrated females at the inlet would indicate daily spawning. However, if the same females were spawning daily at the inlet, they should have had 24 h POFs, which none of them did. Females with 24 h POFs were collected in other zones in the PM (Fig. 7). Thus, although there appears to be daily spawning at the inlet, it is not by the same individuals. Although less obvious, there were also spatial–temporal interactions in the estuarine zones, as the proportion of spawners was not the same in AM and PM samples. A higher percentage of spawners was observed in PM samples (24 %) than in AM sam-
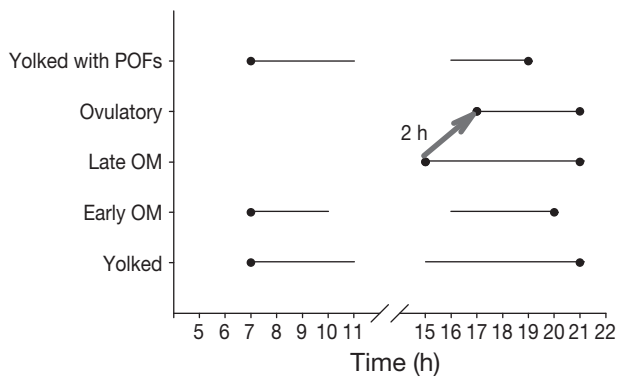
Fig. 5. *Cynoscion nebulosus*. Occurrence of histological indicators by hour of capture (DST). Minimal sampling occurred between 12:00 and 14:00 h; these data are not presented. Histological indicators of spawning included early oocyte maturation (OM), late OM, ovulation, and postovulatory follicles (POFs). 'Yolked' indicated females in the spawning population with no spawning indicators. See text for explanation of arrow
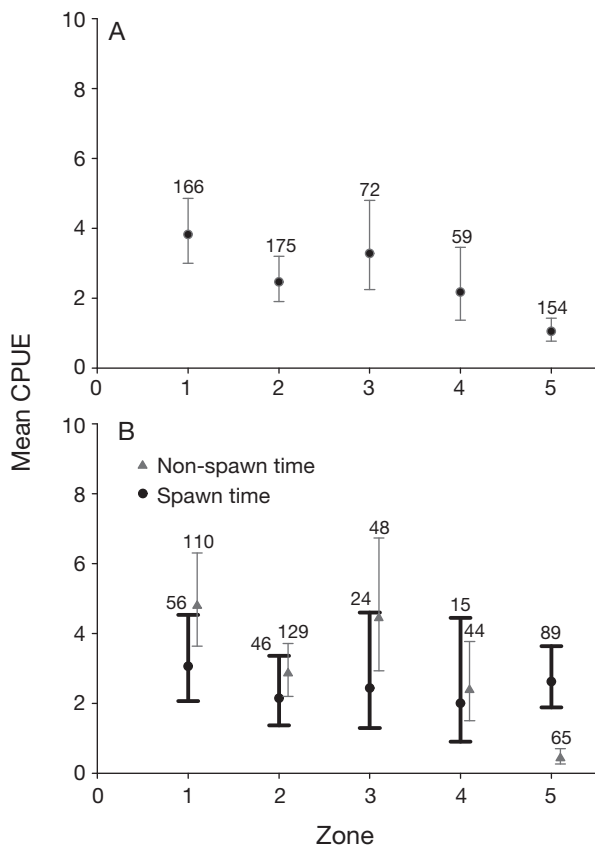

Fig. 6. *Cynoscion nebulosus*. Mean number of spotted seatrout caught per unit effort (CPUE) ± 1 SE by zone for: (A) all sampling events with no temporal filter and (B) for spawn-time sampling events (spawning possible based on seasonal and diel cycles) and non-spawn-time events. CPUE is based on back-transformed least-squared means derived from negative binomial regression. Sample size is indicated above each bar. Zones 1 to 4: estuarine; Zone 5: inlet

ples (15%) in estuarine zones. This difference was significantly related to the time of sampling (binomial regression, n = 455, p = 0.03), but not to gear (p = 0.62) or zone (p = 0.54).

Demographics

Temporal and spatial interactions affected the proportion of mature fish within the study area. Estimated female size at 50% maturity was 261.7 mm TL (SE = 1.96), and 100% were mature by 320 mm TL. Estimated male size at 50% maturity was 226.7 mm TL (SE = 2.32), and 100% were mature by 300 mm TL. Virtually all females matured at Age 1, with no mature Age 0 females and 99.5% mature Age 2 females. Developing Age 1 females (i.e. first-time spawners) were collected throughout the spawning season (Fig. 8), indicating that recruitment to the spawning population is not synchronous. A small number of immature females were collected in all estuarine zones throughout the spawning season: (10% immature, n = 455), but no immature fish were collected at the inlet (Zone 5, n = 170).

First-time spawners (Age 1) had a lower probability of spawning throughout the spawning season than did older females. The percentage of mature females recruited to the spawning population differed significantly by age (binomial regression, n = 507, p < 0.0001) and zone (p = 0.0003). Although the predicted percentage of females in the spawning population was high for Age Classes 2 to 5 (91 to 97%), it was only 73% for first-time spawners. Similar results were found according to size class, with significant effects including size (p < 0.0001), zone (p < 0.0001), and month (p < 0.0001). The predicted percentages of females recruited to the spawning population were low for the size classes of 250 and 350 mm TL (56 and 84%, respectively), but were 95% or more for all larger females. Months with the lowest predicted percentages were March, August, and September. In both analyses, Zones 1 and 2 had higher proportions of non-spawning females than Zone 5.

Older, larger females had a higher probability of spawning. The percentage of spawning females differed significantly by age (binomial regression, n = 436, p = 0.031), zone (p < 0.0001), and month (p = 0.0003), with the highest percentage occurring in Age Class 4, during June or September, and in Zone 5. The mean size of spawning females was significantly larger than that of spawning-capable females (*t*-test, n = 436, p < 0.0001) and the percentage of spawning females increased with size class (Fig. 9). However, this effect was not significant in the binomial regression (p = 0.28).
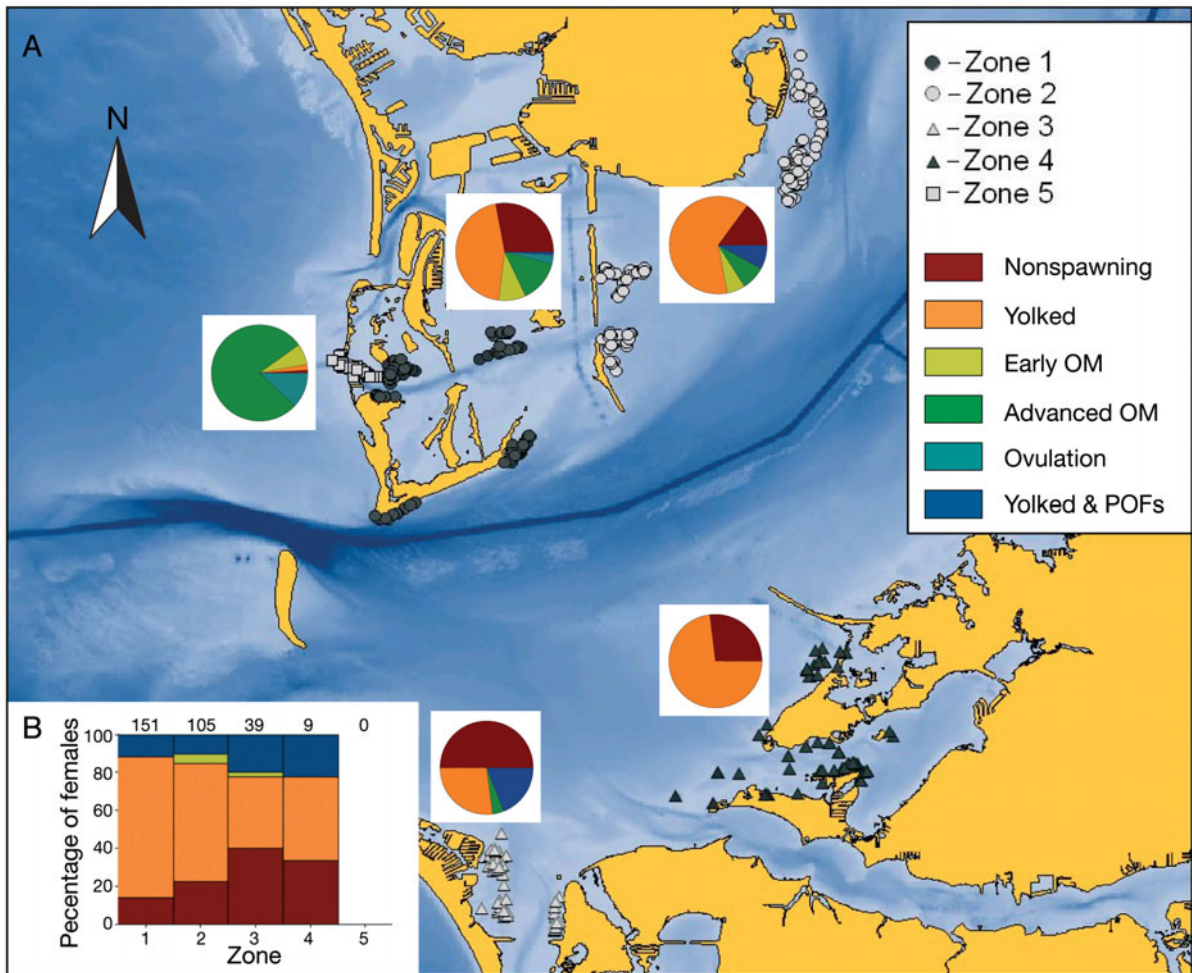
Fig. 7. *Cynoscion nebulosus*. Percentage of mature females collected in the spawning season according to reproductive phase and spawning subphases by zone for: (A) spawn-time events (evening sampling) and (B) morning samples; sample size is indicated above each bar. Zones 1 to 4: estuarine; Zone 5: the inlet. OM: oocyte maturation; POF: postovulatory follicle
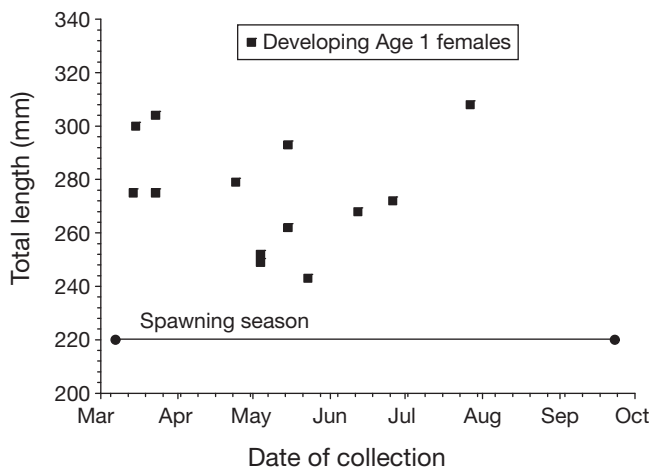

Fig. 8. *Cynoscion nebulosus*. Date of collection and size of developing first-time spawners (Age 1) compared to the population spawning season

Because older, larger fish had a higher probability of spawning, it was difficult to determine if demographics affected spawning site selection (i.e. spawning at the inlet versus in the estuary). The size and age ranges of active spawning phase females at the inlet (Ages 1 to 7; 320 to 615 mm TL; n = 152) were similar to those in Zones 1 and 2 (Ages 2 to 6; 322 to 552 mm TL; n = 16), indicating that there was no threshold size for spawning at the inlet (Fig. 10). However, the mean size of the spawning population females differed significantly by zone ($F_{2,461}$ = 7.43, p = 0.0007), as did age ($F_{2,459}$ = 5.91, p = 0.0029), with females from the inlet being significantly larger (Tukey's honestly significant difference [HSD], p < 0.05) and older (Tukey's HSD, p < 0.05) than those from adjacent estuarine zones. Similarly, the size of spawning condition males differed between zones ($F_{2,512}$ = 9.70, p < 0.0001), as did age ($F_{2,512}$ = 6.56, p = 0.0015), with those from the inlet being signifi-

cantly larger (Tukey's HSD, p < 0.05) and older (Tukey's HSD, p < 0.05).

Larger females produced significantly more eggs per spawning event than smaller females (Fig. 11). Batch fecundities ranged from roughly 87 000 to 1.3 million eggs female^{-1} and significantly increased with both TL (BF = −1 018 285 + 3432.9 TL [r^2 = 0.72, n = 146]), and log$_e$-transformed SW (log$_e$ BF = 4.92 + 1.23 log$_e$ SW [r^2 = 0.72, n = 146]). The predictive batch fecundity relationship with SW was improved by including zone (r^2 = 0.79, p < 0.0001), but not age. Given the clear relationship between batch fecundity and size, even a small difference in size, such as the average size of spawners collected in Zone 1 (384 mm TL) versus the average size of spawners from the inlet (440 mm TL), could almost double egg production (~300 000 eggs versus ~500 000 eggs).

DISCUSSION

Traditionally, reproductive reference points have been based on 2 simple assumptions: (1) that egg production can be predicted based on SSB and (2) that egg production drives reproductive success. However, reproduction is a complex process that occurs over various temporal, spatial, and demographic scales. Although studying each of these factors separately is worthwhile, we chose to address them concurrently because there is a need to understand how coupling across these scales affects egg production and reproductive success.

Fig. 10. *Cynoscion nebulosus*. Size distributions of (A) spawning population females and (B) spawning condition males collected by gill net at the inlet (Zone 5) and in nearby estuarine zones (Zones 1 and 2). Sample sizes are indicated

Egg production

For SSB to be a good predictor of egg production, all mature fish must demonstrate similar reproductive rates throughout their lives. To test this assumption, we assessed whether the percentage *Cynoscion nebulosus* spawning population females remained constant throughout the spawning season and whether this percentage or the percentage of spawners was affected by size or age. In the present study, 23% of females in the spawning season were non-spawning at the time of capture, and 14% were mature, non-spawning. This pattern was driven by 2 aspects of first-time spawners: (1) females did not mature synchronously, but instead continued to recruit to the
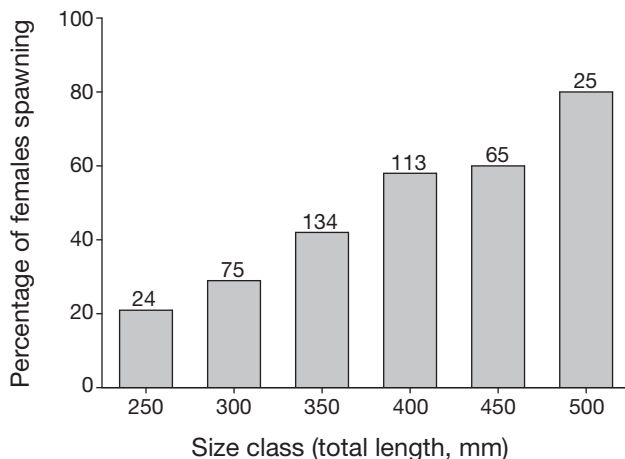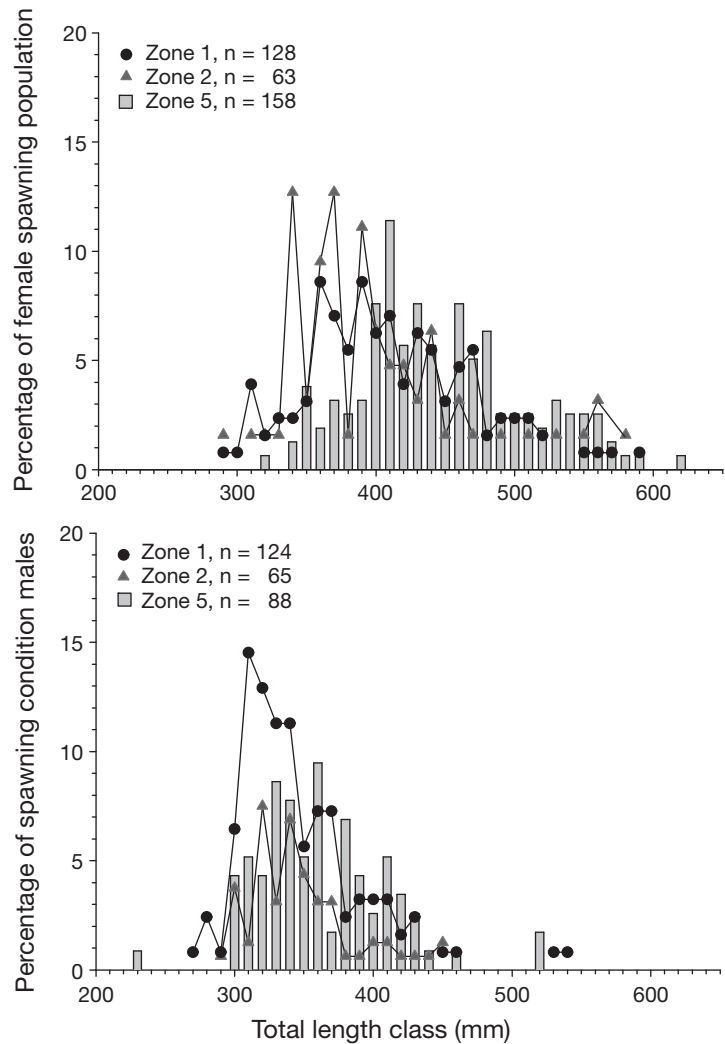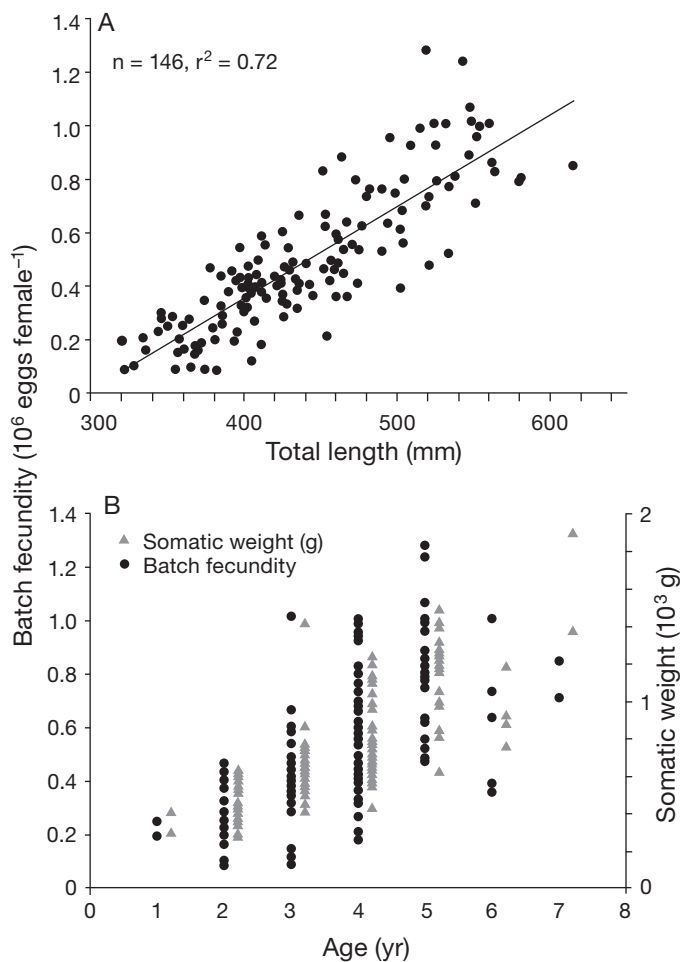

Fig. 9. *Cynoscion nebulosus*. Percentage of female spawners in the spawning population by size category (50 mm total length increments). Sample size is indicated above each bar

Fig. 11. *Cynoscion nebulosus.* Batch fecundity as it relates to size and age. (A) Batch fecundity to total length, with the predicted linear relationship, and (B) individual batch fecundities and somatic weights plotted by age

spawning population throughout much of the spawning season, and (2) first-time spawners had more constricted spawning seasons and spawned less frequently than older females.

The importance of 'skipped spawning' or non-annual spawning has been addressed in recent literature (Jorgensen et al. 2006, Secor 2008). However, non-annual spawning is difficult to prove in the field because we do not yet have the means to track a female's reproductive history throughout a spawning season. For example, for regenerating females collected during the spawning season to indicate non-annual spawning, recrudescence and resorption times of yolked oocytes and spawning indicators would have to be greater than spawning seasonality. For most warm-water species, this is not the case. Similarly, for the occurrence of fish outside their spawning areas during the spawning season to indicate skipped spawning, it must be shown that the time it takes the

fish to migrate between these habitats is greater than the duration of the spawning season. Although this may be true for many species, common snook *Centropomis undecimalis* have demonstrated that they can move 14 km from a spawning aggregation to non-spawning habitat and back again within the spawning season (Lowerre-Barbieri et al. 2003).

While we did not find any indication of non-annual spawning, we did observe differences in spawning seasonality and probability of spawning. First-time spawners had a more restricted spawning season than did older females. The ability of larger, older females to spawn over more extended periods has also been reported for Atlantic cod *Gadus morhua* (Lawson & Rose 2000) and Argentine hake *Merluccisus hubbsi* (Macchi et al. 2004). In other marine species, older females have been reported to initiate spawning sooner than younger cohorts (Berkeley et al. 2004). This disproportionate contribution of older, larger females to egg production is called the 'storage effect' (Berkeley et al. 2004, Secor 2008) and potentially also affects the spawning frequency of batch spawners. In the present study the percentage of spawners increased with both age and size. Given the extended spawning season of spotted seatrout, this could greatly affect the SSB to egg production relationship.

Reproductive success

The spatial–temporal factors affecting reproductive success and recruitment are not yet clearly understood (Berkeley et al. 2004). Most marine fishes produce extremely large numbers of small pelagic eggs (Winemiller & Rose 1993), and it has been widely assumed that these high fecundities and their associated dispersive larval stages conferred greater resilience to fishing pressure (Sadovy 2001). However, Hedgecock's (1994) 'sweepstakes hypothesis' suggests that year classes are produced by only a small fraction of the population: those fish that spawn within the appropriate spatial and temporal window for larval survival. Recent spatially explicit reproductive studies appear to uphold this theory, and it has now been proposed that recruitment may be affected as much by the spatial distribution of spawning activity as by the spawning biomass of a stock (Begg & Marteinsdottir 2002).

If there is no spatial component to reproductive success, then spawning activity should be equally distributed throughout areas with appropriate habitat. We tested this hypothesis by evaluating the level of spawning activity throughout the study area. We found low-intensity spawning activity distributed over a fairly large geographic area (relative to the population distribution) and found high-intensity spawning activ-

ity at one spatially discrete 'hot spot.' A companion study (Walters et al. 2009) found that spotted seatrout spawning activity was distributed throughout most of Tampa Bay, predominantly in shallow-water habitats near seagrass, similar to the habitats we sampled. Although lower Tampa Bay had the greatest number of spawning sites, the passive acoustic survey found no other spawning sites with acoustic signatures as strong or as consistent as those observed at the inlet (authors' unpubl. data).

Although many spotted seatrout reproductive studies have been conducted (reviewed by Brown-Peterson 2003), high-intensity spawning activity (i.e. true spawning aggregation sites) has not been previously reported. The percentage of either OM or hydrated females (used to estimate spawning frequency) ranged from 7 to 44% in reproductive studies conducted in South Carolina (Roumillat & Brouwer 2004), Georgia (Lowerre-Barbieri et al. 1999), Florida (Crabtree & Adams 1998), Mississippi (Brown-Peterson & Warren 2001), and Louisiana (Nieland et al. 2002). These percentages are well below the percentage we observed at the inlet (97%), which is to be expected, given the relative geographic rarity of high-intensity versus low-intensity spawning activity seen in our study. However, spotted seatrout have been reported to spawn in inlets between barrier islands in Louisiana (Saucier & Baltz 1993), suggesting that the inlet 'hot spot', rather than being an anomaly, may represent habitat that is not usually sampled.

The demographics of spawners at the inlet differed from those of spawners in nearby estuarine zones. However, spatial–temporal interactions made it difficult to determine what caused this pattern. Age and size ranges at the inlet and nearby estuarine zones were similar, indicating that, although fish had to expend energy to move to the inlet site to spawn, this behavior was not limited by a demographic threshold. Although greater numbers of larger, older spawners were sampled at the inlet than in estuarine zones, it is not possible to infer a distinct inlet-spawning population based on these differences because this is the expected pattern at a high-intensity spawning site if larger, older fish spawn more frequently.

It has been suggested that natal homing may drive spawning-site selection (Svedang et al. 2007). If this is the case, then inlet spawners would be expected to demonstrate spawning-site fidelity and act as a separate subpopulation from those fish spawning in the estuary. By default, it would also indicate increased reproductive success at this site (i.e. more offspring survived to return and spawn making this a spawning 'hot spot'). Telemetry studies have the potential to address these issues (Robichaud & Rose 2003), and preliminary results with this methodology are promis-

ing (authors' unpubl. data). However, further research is necessary to assess natal homing and the contribution of progeny from the inlet to the Tampa Bay population.

Regardless of what drives spawning-site selection, for both low- and high-intensity spawning sites to persist, there must be a fitness advantage associated with each of these behaviors. In our study, fishes in the low-intensity estuarine zones spawned in what is assumed to be their home range, whereas other fish migrated out of their home ranges to spawn at the inlet. Similarly, dichotomous spawning behavior has been reported for various reef species (Sadovy 1996) and Atlantic cod (Svedang et al. 2007). These 2 patterns may represent trade-offs between progeny survivorship and adult mortality. Because fish migrate to spawn at 'hot spots', they must expend more energy and potentially face greater predation risk. In addition, there is increased risk of mortality at the site, given the large numbers of fish at a discrete location.

Summary

There is an increasing realization that our ability to manage stocks will improve when we are better able to understand a species' reproductive strategy and the aspects that drive reproductive success (Rowe & Hutchings 2003, Berkeley et al. 2004). In the present study, we found that: (1) demographics affected egg production by influencing spawning seasonality, probability of spawning, and batch fecundity; (2) temporal and spatial interactions affected our ability to accurately estimate reproductive parameters, especially spawning frequency; and (3) the spatial distribution of spawning activity was neither random nor evenly distributed over potential spawning habitat, indicating that not all spawning sites were equal.

These results have important management implications. It has been suggested that the resilience of a stock to fishing pressure may be correlated with intraspecific diversity (Frank & Brickman 2001). Thus, we need to evaluate reproductive strategies in terms of dispersion and potential bottlenecks over time, space, and demographics. The spotted seatrout reproductive strategy, in general, is well-dispersed over time and space. Spotted seatrout mature early, have an extended spawning season, and spawn over a large geographic area—although they may be spatially vulnerable at spawning 'hot spots.' Demographically, they will be sensitive to age truncation. Because older, larger females contribute disproportionately to egg production, juvenesced spawning populations will be more vulnerable to recruitment overfishing than SSB-based reference points indicate.

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