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The Red Drum (*Sciaenops ocellatus*) spawning population in the eastern Gulf of Mexico: composition, site fidelity, and size

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Introduction:

Red drum supports one of the largest and most popular fisheries in the southeastern United States. Historically, annual landings in the Gulf of Mexico (GOM) varied between 1 and 3 million pounds until the mid-1980s, when "blackened redfish" became popular increasing demand for this species. Federal waters in the GOM were subsequently closed to harvest for both commercial (1987) and recreational (1988) sectors and have remained closed since (Porch 2000). Inshore fishing effort on juveniles and sub-adults remains high throughout state waters in the GOM (Goodyear 1996). In its latest assessment, the GOM red drum stock was classified as "overfished" (Porch 2000). However, the assessment was limited, in large part, by the lack of data for the offshore adult population, stemming from the federal closure of the adult fishery.

Data Description

The study was conducted from 1 September 2012 to 31 December 2014 in nearshore Gulf of Mexico waters off central Florida (Fig. 1), and monitored waters from shore to approximately 11 km offshore, with a northern border of John's Pass, just north of Tampa Bay and a southern border of Redfish Pass, just south of Charlotte Harbor. Both Tampa Bay and Charlotte Harbor are important nursery areas for red drum and nearshore waters off of Tampa Bay are known as an area where red drum aggregate to spawn in the fall (Murphy and Crabtree 2001, Patterson et al. 2004).

Aerial surveys. Fishery-independent aerial surveys were conducted approximately weekly during the fall spawning seasons of 2012 through 2014 to assess nearshore gulf waters for red drum aggregations (Table 1). These surveys were conducted with a Cessna 172 aircraft and followed a similar protocol to that reported by Powers et al. (2012). The plane's altitude was kept at approximately 1,500 feet and the airplane flew a north/south transect within the study site. The southern transect was flown approximately 3.7 km from land and the northern (return) transect was flown 7.4 km from land to ensure the survey area encompassed the nearshore waters most likely to have aggregations (Murphy and Crabtree 2001; Switzer et al. 2009). Traveling speed of the spotter plane was approximately 80 knots. However, when aggregations were spotted, the plane circled over it at an elevation of approximately 500 to 700 feet, allowing scientists to record GPS location and to better photograph the aggregation and other associated aquatic animals (e.g., sharks, dolphins, permit, jacks). Additional red drum aggregations were sighted during these flights, and they were also photographed and their location noted. However, they were not included in the total number of aggregations detected by the survey.

Purse-seine sampling. A total of 9,089 red drum (8,888 unique individuals) were non-lethally sampled for size, sex, spawning condition, and genetics during the fall spawning seasons of 2012, 2013, and 2014 (Table 2). Samples from red drum aggregations in Tampa Bay coastal waters were captured by a chartered purse boat with experienced captain and crew working in tandem with a contracted "spotter" pilot (Murphy and Crabtree 2001; Winner et al. 2014). The purse net was 639.8 m long and 12.2 m deep. Net sets occurred between 1000 and 1230 h (Eastern Standard Time) and pursing the net took approximately an hour, after which biologists boarded the purse boat and set up special processing stations for non-lethal sampling. Stations were made up of "holding" bins covered with water proof tarps and filled with pumped seawater, with ramps which led to "slides" of heavy duty rubber that ended in measuring cradles and

removable gates. Fish were held loosely in the purse seine while smaller numbers were removed with the brail and transferred to the processing tables throughout the sampling day. After fish were processed, the gate was lifted and fish released. Fish were measured to the nearest mm for both standard length (SL) and total length (TL). Sex was determined based on a combination of strip spawning, sex-specific characteristics, and ovarian biopsies. Because male red drum make courtship sounds (Lowerre-Barbieri et al. 2008), drumming was used as an indicator, as was the presence of milt. Fish without male characteristics were assumed to be female and confirmed by ovarian biopsy and/or ovarian parasites emerging from urogenital pores (Bakenhaster et al. 2014). Fish without male characteristics and urogenital pores too small to be biopsied were assumed to be immature females. Forty-four fish do not have a sex assigned due to recording error or because they were released by mistake prior to taking a biopsy sample. Ovarian biopsies were taken with a catheter composed of a 10 cc syringe equipped with an adapter and tygon tubing with an inner diameter of 1.6 mm. The tubing was inserted 10-20 mm into the urogenital pore and the plunger of the syringe extended to create a vacuum to extract oocytes. All mature females were biopsied in 2012 and 2013 and tissues were preserved for histological analysis. All slides were analyzed in 2012, but due to the large sample size every other female was analyzed histologically for reproductive state in 2013. In 2014, all fish were biopsied to determine sex, but tissue was preserved for histological analysis for every other fish. A portion of the caudal fin was clipped from each fish for genetic analysis. These samples were kept in a cooler on ice until transferred at the laboratory to a -80^0 C freezer.

All gonadal tissue used for histological analysis was processed at the laboratory as follows: fixed in 10% neutrally buffered formalin for a minimum of 24 h, rinsed in water, and stored in 70% ethanol. Samples were embedded in glycol methacrylate, sectioned to 3–5-µm thickness, stained with periodic acid–Schiff's hematoxylin, and then counterstained with metanil yellow (Quintero-Hunter et al., 1991). Germ cell developmental stages, reproductive state, and reproductive phases were assigned based on (Lowerre-Barbieri et al., 2009) and (Brown-Peterson et al., 2011). The following histological indicators were used in females: primary growth (PG), cortical alveoli (CA), vitellogenic (Vtg1-3), and oocyte maturation (OM) stage oocytes and post ovulatory follicles (POFs). Fish with secondary growth oocytes (SG) were considered mature (Lowerre-Barbieri et al., 2011). Because it was difficult to identify early GVM or late stage POFs in biopsy samples, spawning females were identified based on late OM, ovulation, or fresh POFs in both sacrificed and nonlethal samples. Criterion were developed to distinguish POFs from oocyte which were ripped out of their follicles due to the biopsy (Fig. 2)

Genetic tracking:

Genotyping protocols. Total genomic DNA was extracted using the Gentra Puregene Tissue Kit (Gentra Systems, Inc.), according to the manufacturer's directions. DNA quantity and purity was assessed using a NanoDrop 800 spectrophotometer; concentrations were adjusted to ~100 ng/µl. Alleles at microsatellite loci Soc49, Soc85, Soc99, and Soc243 were co-amplified in one multiplex; alleles at microsatellite loci Soc83, Soc129, Soc133, Soc204, and Soc276 were co-amplified in a separate multiplex. Original locus information is given in Turner et al. (1998), including primer sequences, repeat motifs, and Genbank identifiers. Reactions were performed in 12 µl volumes and consisted of ~100 ng genomic DNA, 2.2 mM MgCl2, 0.2 mM of each deoxynucleotide triphosphate, 0.05-0.17 µM each of labeled and unlabeled primers, 5× Promega buffer, and 0.364 units of GoTaq Flexi DNA Polymerase (Promega). The following reaction

profile was used for both multiplexes: 94° C for 1 min; 35 × (94° C for 30 sec, 57° C for 40 sec, and 72° C for 45 sec); 72° C for 30 sec. Following PCR, individual mixtures containing 1.0 µL of the reaction product, 12.5 µL Hi-Di formamide, and 0.15 µL GSROX500 were denatured (95°C for 4 min) and snap-cooled. Fragment analyses were conducted on a single 3130XL Genetic Analyzer (Applied Biosystems) and chromatographic data were converted to genotype data using *GENEMAPPER* software v4.0. To avoid ambiguity, 'genotype' is referred to throughout as the bi-allelic composition of a single locus and 'DNA profile' as the aggregate representation of all loci.

Negative-control PCR reactions were included in all plates. Lab protocol required that any plate or individual reaction showing signs of contamination be repeated. To minimize specimen mistypings caused by allele dropout, homozygous genotypes having peak amplitudes below a predetermined threshold were not scored. One attempt was made to resolve non-scored loci via re-assay. To further mitigate possible mistyping occurrences caused by binning errors, false peaks (i.e., non-detected contamination or PCR-generated artefacts) and allele dropouts, lab protocol required that any pair of DNA profiles differing by genotype scores at either one or two loci (in one or both alleles) be re-assayed and that any inconsistencies be documented and resolved through replicated consensus assays. These planned re-assay results were used to update the rate of specimen mistyping, Ɛ, previously established in FWRI red drum studies.

Individual assignment procedures. The Excel add-in *MS TOOLS* (Park 2001) was used to identify matching sets of DNA profiles and compute numbers of alleles per locus and levels of observed heterozygosity. Genetic effective population size, N_e, was estimated using the single-sample linkage-disequilibrium estimator of Burrows (Hill 1981; Waples and Do 2010) as implemented in *NE ESTIMATOR* v2.01 (Do et al. 2014). Confidence intervals were determined using the parametric option. Given the number of DNA profiles surveyed, significant bias associated with low-frequency alleles was not expected, so estimates were generated by considering all observed alleles. Redundant DNA profiles were culled prior to estimation of N_e.

Implementing the maximum likelihood approach of Kalinowski and Tapir (2006), the program *ML-NULLFREQ* was used to test genotype data for homozygote excesses and to jointly estimate allele frequencies, null frequencies, and genotyping failure rates (β) due to other causes (miscalling). Occurrence rates of missing data were tested for uniformity over loci with a chi-square homogeneity test, as implemented in the program *DROPOUT* v2.3.1 (McKelvey and Schwartz 2005). If the overall chi-square statistic was significant ($p < 0.05$), individual locus contributions were tested for significance using Bonferroni adjusted confidence intervals. Atypical rates of missing data may signal systemic error in affected loci – i.e., null alleles stemming from primer binding-site mutations. DNA profile data were subjected to the "examination of bimodality" (EB) screening procedure (McKelvey and Schwartz 2004) using *DROPOUT*. This procedure was conceptualized on the bell-shaped relatedness distribution expected for large outbred populations (Rousset 2002). If DNA profile pairs from such a population were analyzed with sufficient assignment accuracy in the absence of significant genotyping error, the minimum numbers of loci over which DNA profiles differ should conform to a unimodal distribution. If a bimodal distribution is observed, the first mode is expected to contain mistyped profiles, allowing for targeted error assessment.

For all pairs of DNA profiles, a Bayesian genealogical approach was used to estimate posterior probability for the categorical relationship of identity, *ID*, given the genetic evidence, *E* and appropriate conditioning information *I*. In this approach, the statistical framework for $P[ID|E,I]$ is generalized beyond that of standard formulations for 'probability of identity' (e.g., Paetkau and Strobeck1994, Paetkau et al. 1995) such that the 'close-relative' conundrum (Waits et al. 2001) is addressed analytically rather than by disjunctive means. It was assumed within the model structure that loci are codominant and independent and that genotypes conform to Hardy-Weinberg equilibrium expectations. For all calculation of the likelihood function $P[E|ID]$, allele frequencies were obtained using the maximum likelihood estimator of Kalinowski and Taper (2006). Because (n_p(n_p–1)/2) DNA profile comparisons were conducted over a large pool of n_p DNA profiles in this study, $P[E|ID]$ was conditioned on a heuristic distribution of sampling probabilities for rival categorical relationships using an empirical Bayes procedure. The full rationale for model generalization, as well as estimation and updating procedures, are described elsewhere (Tringali, *manuscript in prep*; see also Tringali 2006). Estimates of $P[ID|E,I]$ and resultant maximum *a posteriori* (*MAP*) classifications represent measures of plausibility that, unlike those from standard formulations, maintain for the pairwise sampling design of a capture-recapture study. Computations were conducted using source R code and implemented in the R program (R Development Core Team 2011).

Acoustic telemetry. Acoustic telemetry is being used to assess the spatial ecology of red drum and how this will affect assumptions used in tag-recapture models to estimate population size. A total of 102 red drum (51 male and 51 females) were acoustically tagged: 60 fish in 2012 and 42 fish in 2013. All fish were intra-peritoneally implanted with acoustic tags (Vemco, 69 KHz V16TP-6H) and released within the Tampa Bay nearshore waters. Three receiver arrays were deployed to monitor these fish: one in the sampling area off of Tampa Bay (TB array), the other off the nearest estuarine neighbor, Charlotte Harbor (CH array) and a third high-density array embedded within the TB array to test the efficacy of the larger array (Fig. 1). The Tampa Bay array had 33 receivers (VR2-Ws, Vemco Ltd, Shad Bay, NS, Canada); 20 located at previously identified red drum aggregation sites and 13 to fill in gaps, primarily in the southern area. Because there was no similar data on red drum aggregation sites for the Charlotte Harbor spawning area, 15 receivers were deployed in an evenly spaced grid off this estuary and 7 receivers added at aggregation sites identified in 2012 and 2013. Details of the methods can be found in (Lowerre-Barbieri et al. 2015; Lowerre-Barbieri et al. *in press*)

Data analysis. One fish is missing a SL measurement and another is missing a TL measurement due to recording error. Forty-one fish do not have a sex assigned due to recording error or because they were released by mistake prior to taking a biopsy sample. In the data, these fish were given a "U" (undermined) for sex. Sex ratio was computed from all genotyped individuals with a sex assignment and evaluated against a null hypothesis of equality using a two-tailed, one proportion Z test. Sex-specific capture data were subjected to two-tailed, two proportion Z tests to examine the possible effect of gender on recapture rate**.**

Population size estimation. All tag-recapture models are based on a range of assumptions about immigration/emigration, mixing, survivorship, tag effects on behavior, tag loss, and capture probability. We are using telemetry to assess these assumptions and help inform the development of a POPAN Jolly-Seber model. However, because our telemetry results suggested

relatively closed populations within each spawning season, our preliminary analysis used within season, closed-population estimates for adult red drum made using the full likelihood estimators for the probability of initial capture '*p*' and for recapture '*c*' (Otis et al. 1978). The analysis was implemented using MARK software (Version 8.0, White and Burnham 1999). Individual within-season histories for the period September 17 – October 17, 2013 and for October 9-28, 2014 (Table 5) were analyzed separately to derive estimates of abundance under the assumption of the population being closed, no births/immigration or deaths/emigration, during this time frame. Abundance was derived for capture-recapture models that specified the following three constraints on *p* and *c*: 1) constant through time and $p=c$, 2) constant through time but $p \neq c$, and 3) time-specific and $p=c$. All models estimate a parameter for the number of unseen fish in the population, *fo*. Model comparisons within each season were made using the Akaike information criterion correction for finite population sizes, AICc.

Results / Discussion:

Aerial surveys. A total of 29 red drum aggregations were detected within our transects during aerial surveys. Red drum aggregations were detected more often off of Tampa Bay, but also occurred off of Charlotte Harbor. The number of red drum aggregations spotted on any given date varied (range: 0 to 5), as did their location and size (Fig. 3). In 2012, aerial surveys were conducted from 28 September to 19 November. In consequent years, aerial surveys began in mid-August as fish were sighted on the first September flight in 2012 and preliminary data indicated some acoustically-tagged red drum returned to Tampa Bay nearshore waters in August.

The distribution and number of aggregations detected also differed between spawning seasons. In 2012, when there was a strong red tide off of Charlotte Harbor, aggregations were detected only in Tampa Bay nearshore waters. But in consequent years they were also detected off of Charlotte Harbor. Although the number and range of dates sampled were similar in 2013 and 2014, twenty-four aggregations were detected in 2013 and only six in 2014.

Composition of aggregations. The number of red drum sampled on any given date ranged from 109 to 1,038 fish and depended on a number of factors including how the net was set and if boaters were in the area, as well as more rapid processing times as experience increased. Only on one date did the spotter plane pilot estimate that most, if not all, of the aggregation was caught (11/22/2014). Often the aggregation would split up as the net was being set. All processed fish were released alive, although the remains of one fish in 2013 were recovered after what appeared to be a shark attack and this fish was removed from the data set. In 2013, on four dates there were more red drum than could be worked up and unsampled fish were released alive by simply opening the mouth of the purse seine after sampling had been completed (approximately 400-500 per day). Similarly on three out of four sampling dates in 2014, unsampled fish were released alive. On 10/9/2014 an estimated 500 were released, 50 on 10/22/2015, and 1,000 on 10/28/2014. Eighteen fish died on the last date in the net due to the very large catch (> 2,000 fish) and the long processing time.

Of the 8,888 unique fish sampled (i.e., multiple measurements on recaptures were not included), 3,894 were female and 4,953 were male. Sex could not be assigned for forty-one fish and due to a recording error one male did not have a TL measurement. In each of the three years, the

overall sex ratio was skewed slightly towards males (1.3:1 M to F). Fish ranged in size (Fig. 4) from 541 mm to 1099 mm total length (TL) and males were slightly smaller (mean=897 mm TL) than females (mean=912 mm TL). The size of fish sampled was quite similar over the three years (Table 3). Mean size differed significantly by sex (GLM, n=8,845, $P<0.0001$) and date sampled ($P < 0.0001$) and there was a significant interaction ($P=0.0003$). Both sexes exhibited a number of small outliers presumably associated with young fish recruiting to the spawning population for the first time.

Ovarian biopsies were assessed histologically for 2,378 females. Spawning fraction (proportion of mature females which would have spawned that day based on the presence of hydrated oocytes) varied significantly by date in all three years. (Table 4). The proportion of actively spawning females per sampling date varied from 1% to 100% over the three years, indicating that although fish spawned in the sampling area, it could not be assumed that presence on the spawning grounds meant an individual was spawning on that date.

Recaptures. One hundred ninety-nine unique fish were recaptured over the three year study period and all Bayesian genealogical-model assumptions were satisfied; with assigned individuals shown to be robust against Type I and II error. Two fish (one male and one female) were recaptured twice over this time period. In 2012 a total of 1,849 fish were sampled by purse seine from 5 October to 17 October and there was only one within-season recapture (Table 2). In 2013, 3,421 fish were sampled and there were 23 within-season recaptures and 25 recaptures from fish first captured in 2012. In 2014, purse seine sets were closer together than in past years and a total of 3,618 fish were sampled. Within-season recaptures were higher than in other years with (n=95), as were recaptures from previous years (n=48). Mixing amongst aggregations was evident as there were only two dates when recaptures came from just one aggregation and they were very early in the study (10/17/2012 and 9/17/2013). By the last year of the study, recaptures on all sampling dates came from a range of original aggregations, ranging in number from four to ten, and increasing as the season progressed.

The null hypothesis that the overall sex ratio (1.37:1) of the 199 recaptured fish equated to the ratio of all genotyped fish could not be rejected ($Z = -0.665$; $p = 0.5029$). The null hypothesis that the sex ratio (1.29:1) of the 119 fish recaptured within the same sampling season equates to the sex ratio of all genotyped fish could not be rejected ($Z = 0.068$; $p = 0.7937$). Again, the null hypothesis that the sex ratio (1.47:1) of the 84 fish recaptured across sampling seasons equates to the sex ratio of all genotyped fish could not be rejected ($Z = -0.806$; $p = 0.4179$). Thus, based on direct testing, there was no evidence of gender-based heterogeneity in recapture rates.

Population structure.

A total of seventy-one fish were detected on dates after their implant date and five fish were presumed dead, when their tags were determined as stationary. Red drum occurred in nearshore waters off of Tampa Bay, primarily during the spawning season from August to November, with some fish still present in December (Lowerre-Barbieri et al. 2015; Lowerre-Barbieri et al. *in press*). Most of the fish detected in the Tampa Bay array (91%, n=68 fish) were detected only during the months of August through December. We consider this time period to be the reproductive period, i.e., it is larger than the spawning season, including the time when fish move

to the spawning grounds in preparation to spawn and a recovery period after spawning before leaving the spawning grounds. Although several fish were detected outside of the reproductive period in the Tampa Bay array (n=5) all of these fish were also detected within the reproductive period in at least one year. Only one fish was detected on more dates outside of the reproductive period than within it, suggesting that this area covered by the TB array is part of its home range. In contrast, other detections outside of the reproductive period were either of short duration, suggesting fish were moving through the area, or just prior to the reproductive period.

Although there was relatively strong spawning site fidelity from one year to the next to the Tampa Bay spawning site, there was also some apparent mixing with the Charlotte Harbor spawning site (Fig. 5). Of the sixty fish implanted in 2012, three were identified as mortalities. Of the 57 fish which were assumed to be alive, 61% (n=35) were detected in 2012. Of these 35 fish, 25 were detected in the 2013 reproductive period, and 19 in the 2014 reproductive period. In the 2013 reproductive period, most (n=21) of these fish were detected in the TB array but four fish were assumed to have spawned off of Charlotte Harbor given their detection pattern (detected on more dates in the CH array than in the TB array or only in the CH array). In 2014, one of these four fish was not detected and the other three again appeared to spawn off Charlotte Harbor. One additional fish, which was detected in the TB array in 2013, also exhibited a detection pattern suggesting in 214 it spawned off of Charlotte Harbor. Fish implanted in 2013 showed a similar pattern, with the exception that more of these fish were detected in their implantation year (n=36). Of these 36 fish, 23 were detected in the 2014 reproductive period and all of these demonstrated a detection pattern suggesting they spawned off of Tampa Bay.

Population size estimate.

The models that solved for time-specific capture/recapture probabilities were judged the best model fits to the data using AICc, both having AIC weights approaching 1.0. The estimates for capture/recapture probabilities were lower in 2013 than in 2014 for all sampling events (Table 6). Given the similar number of fish examined each year, this lower probability of capture/recapture resulted in a significantly higher estimate of red drum on the spawning grounds off Tampa Bay during 2013 (~200,000 fish) than in 2014 (~51,000 fish). However, these are simply preliminary estimates to gain insight into the possible population size range. The much lower estimate in 2014 is due to the high recapture rates between Oct 22 and Oct 28. Both of these dates more than 1,000 fish were sampled and the sample sites were fairly close together, increasing the probability of recapture (Fig. 6). Analysis is on-going with the telemetry data to assess the assumptions used in this preliminary analysis and to help inform the development of a POPAN Jolly-Seber open population model.

References

Bakenhaster, M., S. Lowerre-Barbieri, Y. Kiryu, S. Walters, and E. Fajer-Avila. 2014. *Philometra floridensis* (Nematoda: Philometridae) damages ovarian tissue without reducing host (Sciaenops ocellatus) fecundity. Diseases of Aquatic Organisms **108**:227-239.

Brown-Peterson, N., Wyanski, D., Saborido-Rey, F., Macewicz, B., Lowerre-Barbieri, S. (2011) A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries: Dynamics. *Management, and Ecosystem Science [online serial]* **3,** 52-70.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J. and J. R. Ovenden. 2014. NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Molecular Ecology Resources, 14:209-214.

Goodyear, C. 1996. Status ofthe red drum stocks ofthe GulfofMexico: Report for 1996. USDOC, NMFS, SEFC, Miami Laboratory Contribution MIA **95**:96-47.

Hill, W.G. 1981. Estimation of effective population size from data on linkage disequilibrium. Genetical Research Cambridge 38:209-216.

Kalinowski, S. T., M. L. Taper, and S. Creel. 2006. Using DNA from noninvasive samples to identify individuals and census populations: an evidential approach tolerant of genotyping errors. Conservation Genetics, 7:319–329.

Lowerre-Barbieri, S.K., Barbieri, L.R., Flanders, J.R., Woodward, A.G., Cotton, C.F., Knowlton, M.K. (2008) Use of Passive Acoustics to Determine Red Drum Spawning in Georgia Waters. *Transactions of the American Fisheries Society* **137,** 562-575.

Lowerre-Barbieri, S.K., Henderson, N., Llopiz, J., Walters, S., Bickford, J., Muller, R. (2009) Defining a spawning population(Spotted Seatrout *Cynoscion nebulosus*) over temporal, spatial, and demographic scales. *Marine Ecology Progress Series* **394,** 231-245.

Lowerre-Barbieri, S., Ganias, K., Saborido-Rey, F., Murua, H., Hunter, J. (2011) Reproductive timing in marine fishes: variability, temporal scales, and methods. Marine and Coastal Fisheries: Dynamics. *Management, and Ecosystem Science [online serial]* **3,** 71-91.

Lowerre-Barbieri, S., Tringali, M., Murphy, M., Winner, B., Porch, C. (2015) Assessing Red Drum spawning stock size and structure in the eastern Gulf of Mexico. CRP Final Report. 30 pp.

Lowerre-Barbieri, S., S. Walters-Burnsed, J. Bickford. (*in press*). Assessing reproductive behavior important to fisheries management: a case study with red drum, *Sciaenops oscellatus. Ecological applications.* Doi:10.1890/15-0497**.**

McKelvey, K. S., and Schwartz, M. K. 2004. Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. Journal of Wildlife Management 68:439–448.

McKelvey, K. S. and M. K. Schwartz. 2005. DROPOUT: a program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. Molecular Ecology Notes 5:716–718.

Murphy, M.D., Crabtree, R.E. (2001) Changes in the age structure of nearshore adult red drum off west-central Florida related to recruitment and fishing mortality. *North American Journal of Fisheries Management* **21,** 671-678.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monograph 62. 135pp.

Paetkau, D. and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology 3, 489–495.

Paetkau, D., W. Calvert, I. Sterling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4, 347–354.

Park, S. D E. 2001. MS TOOLS. Version 3. [http://acer.gen.tcd.ie/sdepark/ms-toolkit].

Patterson, H., McBride, R., Julien, N. (2004) Population structure of red drum (*Sciaenops ocellatus*) as determined by otolith chemistry. *Marine Biology* **144,** 855-862.

Porch, C.E. (2000) Status of the red drum stocks of the Gulf of Mexico. *Southeast Fisheries Science Center. Sustainable fisheries Division Contribution: SFD-99/00-85. 62p*.

Powers, S.P., Hightower, C.L., Drymon, J.M., Johnson, M.W. (2012) Age composition and distribution of red drum (*Sciaenops ocellatus*) in offshore waters of the north central Gulf of Mexico: an evaluation of a stock under a federal harvest moratorium. *Fishery Bulletin* **110**.

Quintero-Hunter, I., Grier, H., Muscato, M. (1991) Enhancement of histological detail using metanil yellow as counterstain in periodic acid Schiff's hematoxylin staining of glycol methacrylate tissue sections. *Biotechnic & Histochemistry* **66,** 169-172.

Rousset, F. 2002. Inbreeding and relatedness coefficients: what do they measure? Heredity 88:371–380.

Switzer,T., M.D. Murphy, B.L. Winner, K.E. Flaherty, and J. Vecchio. 2009. Final Report to NOAA/NMFS from Florida Fish and Wildlife Conservation Commission for Award NA05NMF4331078, "Size and age structure, and catch-and-release mortality estimates of subadult and adult red drum (*Sciaenops ocellatus*) in the Tampa Bay estuary and nearshore Gulf of Mexico waters". 70 p.

Tringali MD. 2006. A Bayesian approach for the genetic tracking of cultured and released fishes. Fisheries Research 77(2):159-172

Turner, T.E., L.R.Richardson, and J.R. Gold. 1998. Polymorphic microsatellite DNA markers in red drum (*Sciaenops ocellatus*). Molecular Ecology 7:1771-1788.
Waits LP, Luikart G and Taberlet P. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology, 10:249-256.

Waits L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10(1):249-56.

Waples, R. S. and C. Do. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications 3:244–262.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46 Supplement: 120-138.

Winner, B.L., Flaherty-Walia, K.E., Switzer, T.S., Vecchio, J.L. (2014) Multidecadal Evidence of Recovery of Nearshore Red Drum Stocks off West-Central Florida and Connectivity with Inshore

Table 1. Summary of aerial surveys and number and date range of when red drum aggregations were sighted within our transect.

Year	Dates sampled	Date range sampled	Date range with aggregations	Aggregations
2012	10	28 September to 19 November	28 September to 19 November	6
2013	15	19 August to 25 November	26 August to 18 November	17
2014	14	18 August to 21 November	25 August to 29 September	6

Table 2. Summary of recaptured individuals by year, date, and, the original date and aggregations they were sampled from. Dates on the left are all dates that aggregations were sampled by purse seine. Dates across the top reflect sampling dates when fish were recapture. No fish were recaptured on 10/5/2012 or 10/9/2012.

Recapture year	**2012**	**2013**						**2014**				
	Recapture Date											
	10/17	**9/17**	**9/19**	**10/1**	**10/3**	**10/15**	**10/17**	**10/9**	**10/20**	**10/22**	**10/28**	**N**
Sample date												
10/5/12	1		1		1	1	1		2	4	2	**526**
10/9/12				2		3	3	2		2		**688**
10/17/12		1	1	6		2	3				2	**635**
9/17/13			1		1				1		2	**140**
9/19/13				1		5	1	2	2	5	2	**689**
10/1/13						10	4	3	1	3	8	**690**
10/3/13										1		**109**
10/15/13								1	1	4	4	**976**
10/17/13										2	1	**817**
10/9/14									1	10	22	**1019**
10/20/14										32	6	**334**
10/22/14											24	**1138**
10/28/14												**1127**

Table 3. Size of fish sampled by year

Year	Sample size	Mean TL (mm)	Minimum	Maximum	± SD
2012	1834	901.4	576	1064	59.3
2013	3411	909.3	571	1099	57.2
2014	3601	902.9	541	1069	68.1

Table 4. Spawning fraction by date.

Year	Date	Sample size	% hydrated	Annual sample size	P value for Chi square
2012	10/5/2012	210	86%		
2012	10/9/2012	301	1%		
2012	10/17/2012	265	34%	776	<0.0001
2013	9/17/2013	53	9%		
2013	9/19/2013	152	7%		
2013	10/1/2013	177	9%		
2013	10/3/2013	70	4%		
2013	10/15/2013	161	75%		
2013	10/17/2013	155	100%	768	< 0.0001
2014	10/9/2014	238	70%		
2014	10/20/2014	113	78%		
2014	10/22/2014	273	85%		
2014	10/28/2014	210	59%	834	<0.0001

Table 5. Individual capture history frequencies for purse-seine captured red drum that were individually identified by genetics and with an assigned sex. Sampling dates are given for each position in the capture history with '1' indicating a capture and '0' indicating no capture.

2013

Sep 17	Sep 19	Oct 1	Oct 3	Oct 15	Oct 17	Number
1	0	0	0	0	0	138
1	1	0	0	0	0	1
1	0	0	1	0	0	1
0	1	0	0	0	0	681
0	1	1	0	0	0	1
0	1	0	0	1	0	5
0	1	0	0	0	1	1
0	0	1	0	0	0	671
0	0	1	0	1	0	10
0	0	1	0	0	1	4
0	0	0	1	0	0	109
0	0	0	0	1	0	974
0	0	0	0	0	1	816
						3,412

2014

Oct 9	Oct 20	Oct 22	Oct 28	Number
1	0	0	0	983
1	1	0	0	1
1	0	1	0	9
1	0	0	1	22
0	1	0	0	295
0	1	1	0	32
0	1	0	1	
0	0	1	0	1,112
0	0	1	1	24
0	0	0	1	1,117
				3,601

Table 6. Time-specific estimates (and standard error and 95% confidence intervals) for the probability of initial capture *p*, the probability of recapture *c*, and the number of unseen fish during the 2013 and 2014 purse seine sampling periods *fo*. The derived estimate of *N* (*N*-hat) and standard error and confidence interval are also given.

2013			Confidence Interval	
Parameter	Estimate	SE	2.5th	97.5th
p, Sep 17	0.00070	0.00016	0.00045	0.00109
p=*c*, Sep 19	0.00347	0.00073	0.00230	0.00524
p=*c*, Oct 1	0.00345	0.00073	0.00229	0.00522
P=*c*, Oct 3	0.00055	0.00013	0.00035	0.00087
p=*c* Oct 15	0.00498	0.00104	0.00330	0.00750
p=*c*, Oct 17	0.00413	0.00087	0.00274	0.00624
*fo,*not caught	195,224	41,129	129,762	293,713
Derived value				
N-hat	198,636	41,129	133,174	297,125

2014			Confidence Interval	
Parameter	Estimate	SE	2.5th	97.5th
p, Oct 9	0.01977	0.00207	0.01609	0.02426
p=*c*, Oct 20	0.00651	0.00074	0.00520	0.00813
p=*c*, Oct 22	0.02292	0.00239	0.01869	0.02810
p=*c*, Oct 28	0.02277	0.00237	0.01856	0.02791
*fo,*not caught	47,744	5,134	38,694	58,910
Derived value				
N-hat	51,345	5,134	42,295	62,511

Figure 1. Study site location off west central Florida and locations of acoustic receivers deployed as part of the Tampa Bay, Charlotte Harbor, and the high resolution arrays. The high resolution array was deployed based on the location with the highest number of fish detected in 2012 (inset, bubble size represents number of fish). Five additional receivers were added to the Charlotte Harbor site based on red drum aggregations sighted in the aerial survey in 2013.

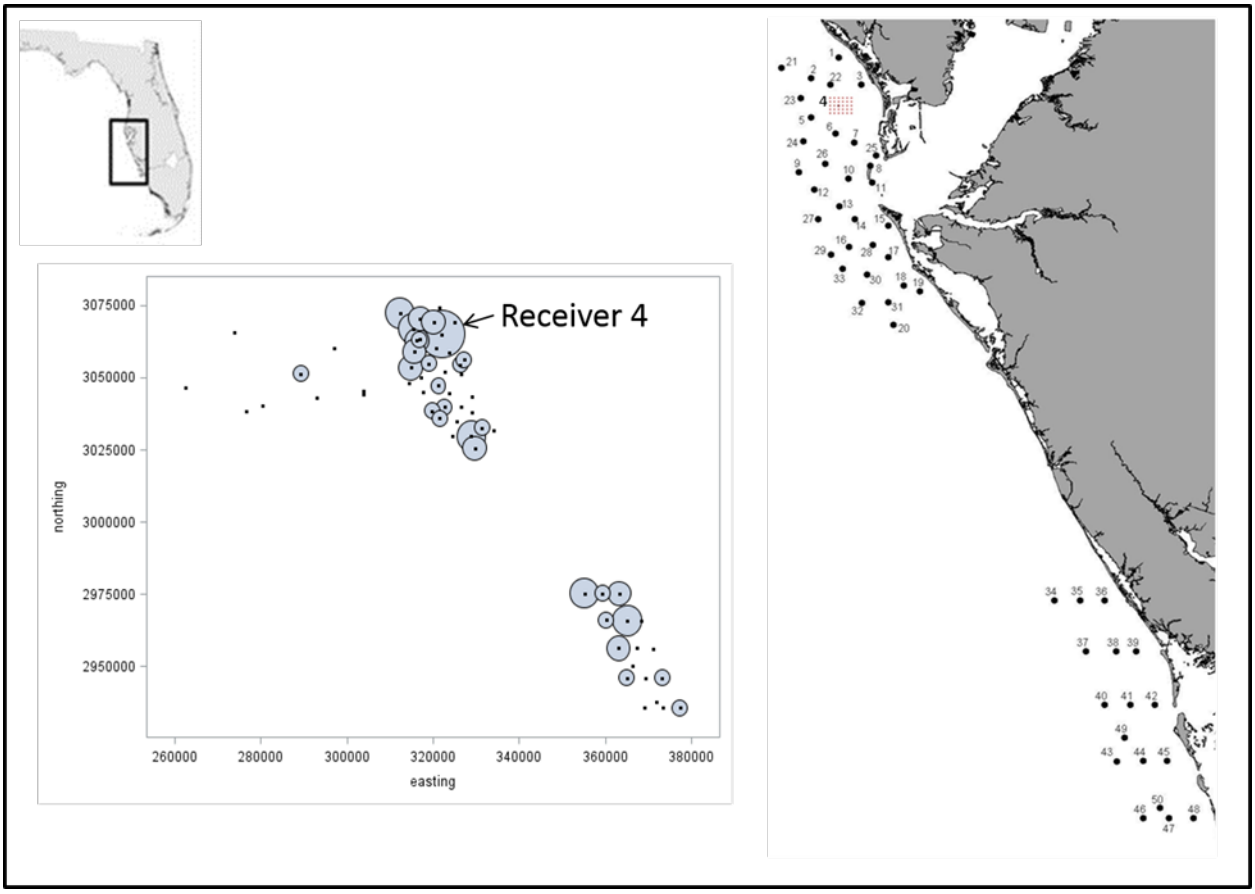

Figure 2. Histological basis for identifying post-ovulatory follicles versus ripped follicles in red drum ovarian biopsies. Note that the ripped follicle has a proliferation of blood cells and it is not possible to identify the thecal, granulosa and basal membrane layers.

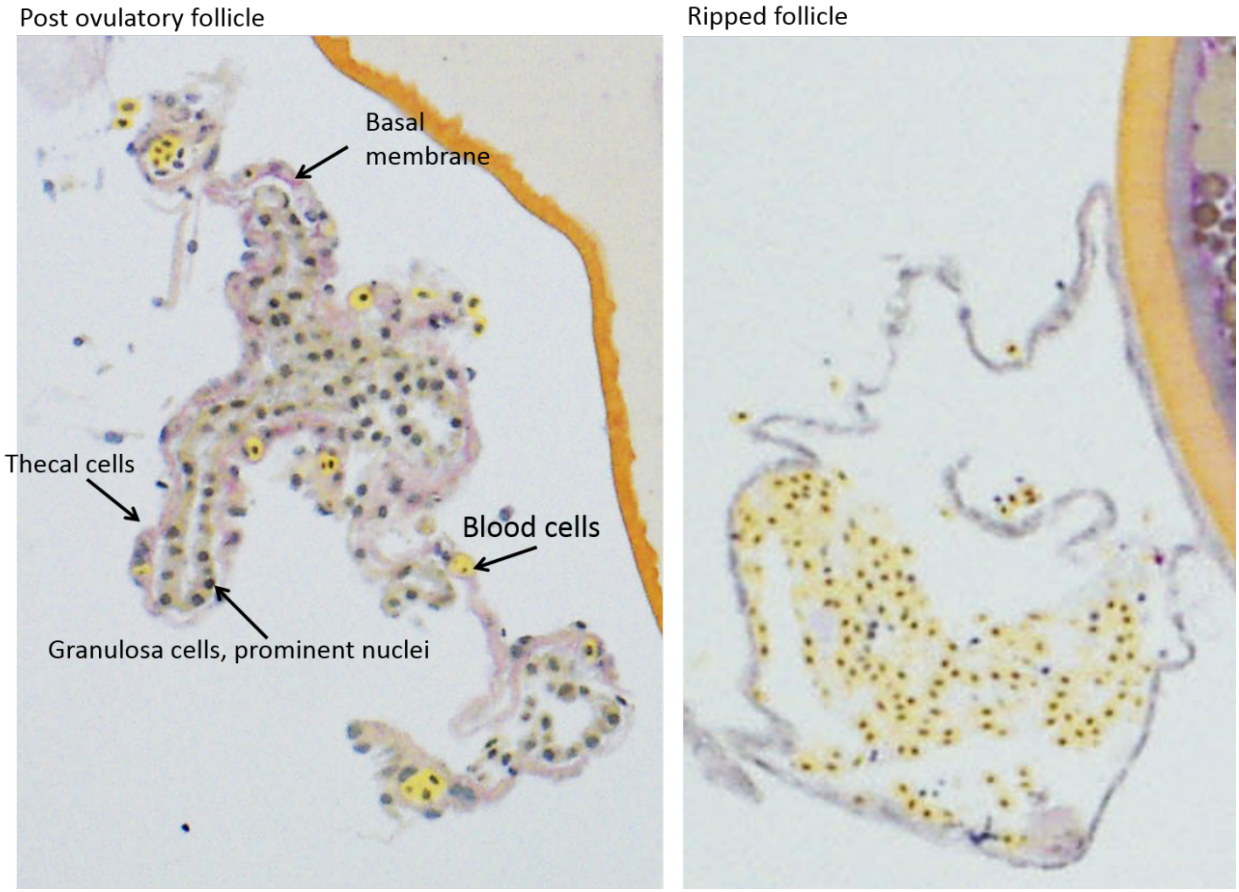

Figure 3. Red drum aggregations detected on the first aerial transect flown on 9/28/2012 (path indicated on inset, shaded area corresponds to the Tampa Bay receiver array). Five aggregations were detected on this date (the location of aggregation B cannot be seen on the map as it falls under that of C). Times that aggregations were sited are to the left of each photograph. Aggregation appearance varied depending on the number of fish, location within the water column and whether they are feeding (B) or reacting to potential predators, in this case dolphins were breaking to the right of the aggregation (E2). Frigate birds were commonly associated with red drum aggregations and can be seen in the lower left corner of D.

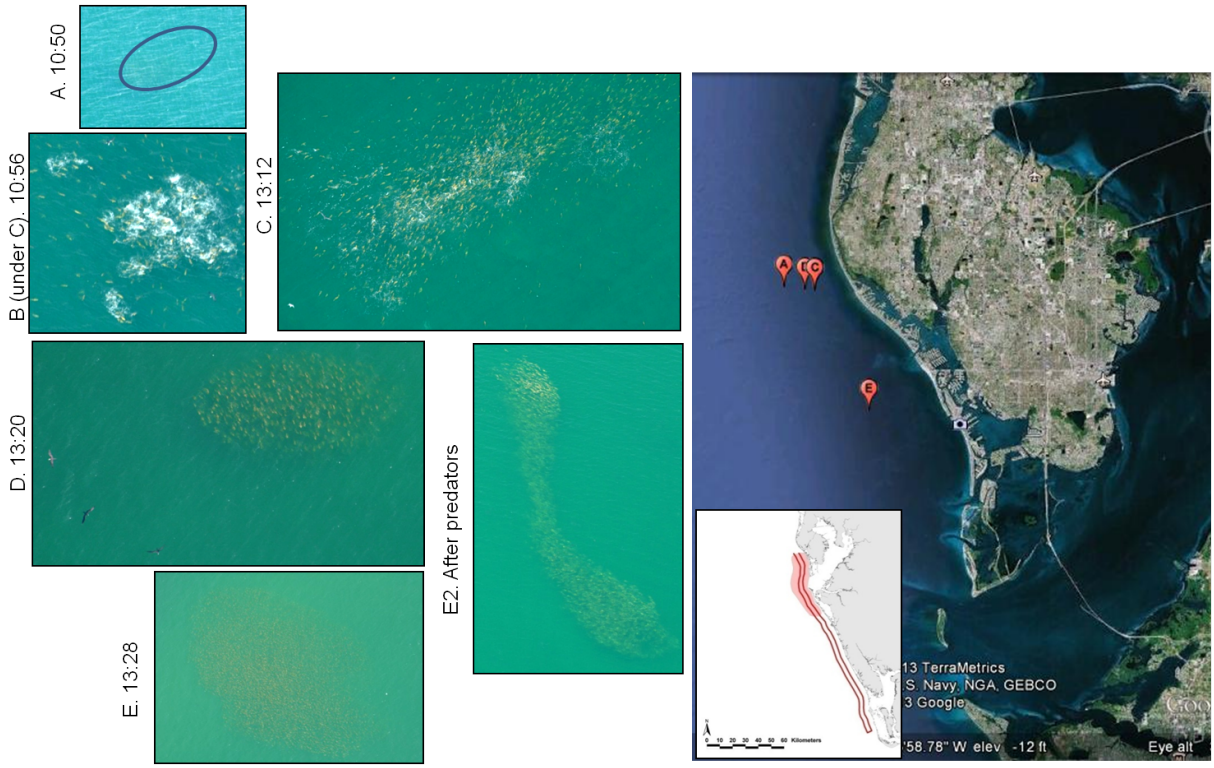

Figure 4. Total length (TL) distributions of females (F) and males (M) samples by purse seine in Tampa Bay nearshore waters. In the box plots, mean is denoted by a diamond and median with a vertical line. The ends of the boxes represent the 25th and 75th percentiles and the whiskers are the minimum and maximum data. Dots represent outliers.

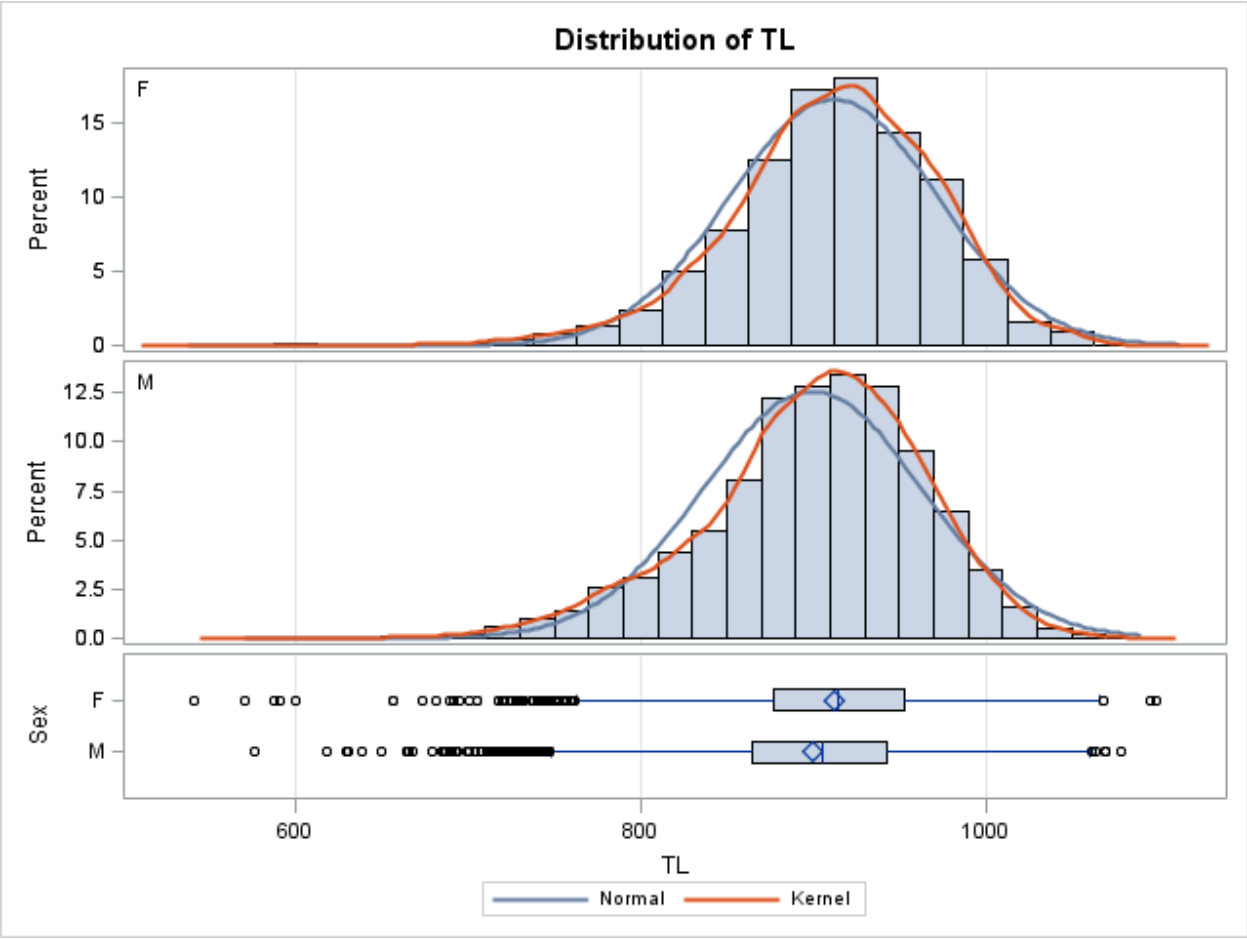

Figure 5. Daily detections of all acoustically tagged red drum. Blue represents dates fish were detected in the Tampa Bay array and red represents dates fish were detected in the Charlotte Harbor array. Individual fish were never detected in both arrays in one day. The lines represent the time period over which purse seine samples were also collected for genetic samples.

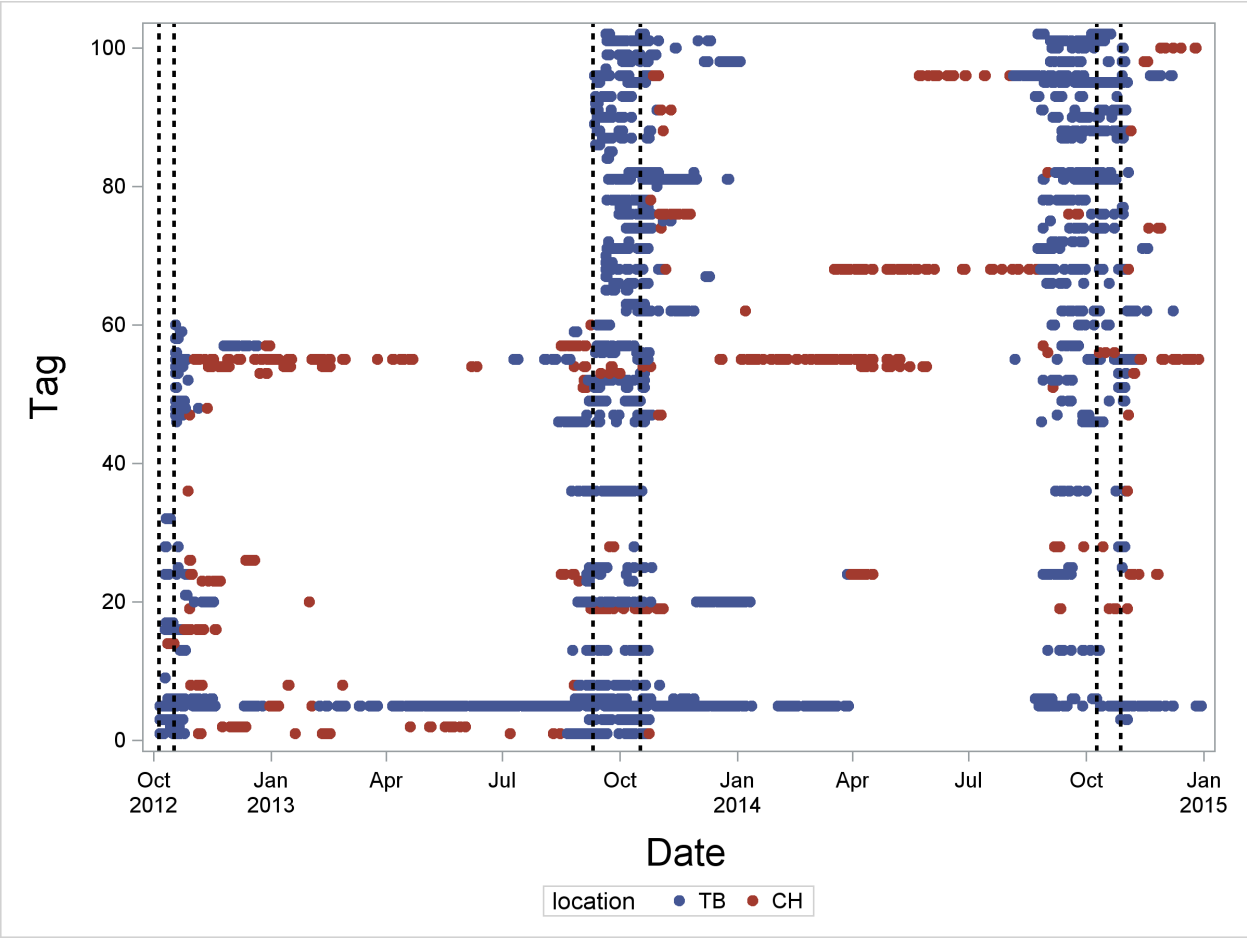

Figure 6. Distribution of receivers in the Tampa Bay array and location of purse seine samples for each year sampled. The red arrow indicates the area where the high resolution array was deployed.