

SETBACKS AND SURPRISES

Attempted use of an uncommon bay scallop color morph for tracking the contribution of restoration efforts to population recovery

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In our ongoing bay scallop restoration efforts in eastern Long Island, NY, United States, we have planted millions of hatchery-reared juveniles to serve as broodstock when they mature. These plantings have driven the resurgence of larval recruitment, benthic population sizes, and commercial fishery landings over a 12-year period. In this article, we detail an attempt to track the contribution of our restoration efforts to population rebuilding using planted “skunks,” an uncommon color morph, by looking for increased skunk frequencies in subsequent wild cohorts. Although we saw higher skunk frequencies among large juveniles in natural populations in the first 3 years after extensive skunk plantings, supporting use of uncommon color morphs as a passive tracer, this conclusion was not supported when examined over a 12-year period, as no strong correlations were seen between skunk frequencies at different life stages (adult broodstock, small and large F1 juveniles, F1 adults) for respective cohorts. Potential reasons for poor correlation between skunk frequencies and restoration efforts include lower than expected production of skunk offspring, dilution of out-planted contribution to growing natural populations, interannual variability in skunk frequencies that may have obscured the expected skunk signal, and/or differentially higher mortality of skunks at postset and larger juvenile stages. In the latter case, skunks experienced higher overwinter mortality, most likely due to predation, in 9 of 11 years. This led us to suspend skunk plantings after 3 years. Nevertheless, commercial fishermen perceived skunks to be “our” scallops, helping raise the profile of and support for our restoration efforts.

Key words: *Argopecten*, bivalve, larval recruitment, overwinter mortality, restoration, shell color

Implications for Practice

- Tracking the success of restoration efforts is critically important for fostering the support of different user groups; however, many of these approaches (e.g. genetic markers, intensive surveys) are expensive or infeasible.
- We utilized a phenotypic marker as an additional, low cost means of supporting strong correlations that we had previously established between overall numbers of planted bay scallop broodstock and both natural recruitment and commercial fishery landings. The present effort ultimately proved unsuccessful over the long term, but may provide guidance for future efforts with other species.
- Despite our lack of success on an operational level, the perception of increased abundance of uncommon color morphs by fishermen has raised support for restoration efforts.

Introduction

Physical, electronic/acoustic, chemical, and biological tags or marks have been used extensively in studies of animal behavior and migration (Leopold 2019), for estimation of mortality, age, and population size (King 2007) and for evaluating the

success of population enhancement and introduction programs (Bell et al. 2005; Wildlife ACT 2019). Biological (natural) tags include coloration and other morphological characteristics, protein banding patterns, DNA sequences, and parasites (Jennings et al. 2001; Marshall & Holmberg 2018); these have been used to differentiate individuals and populations of diverse taxa, including terrestrial vertebrates, fish, and aquatic invertebrates. Among marine species, uncommon to rare color morphs have

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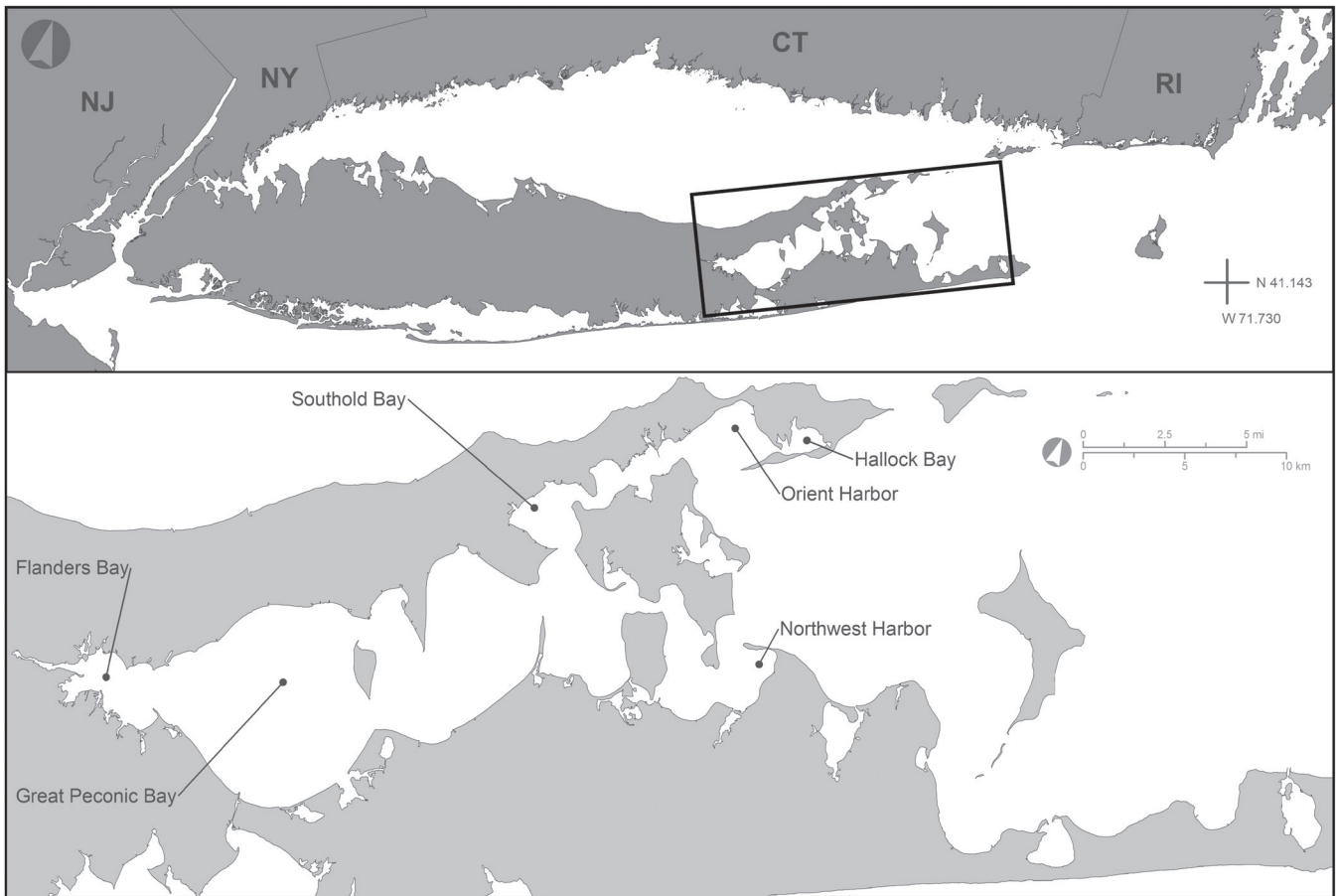


Figure 1. Northeastern United States (NJ: New Jersey, NY: New York, CT: Connecticut, RI: Rhode Island), showing the location of the Peconic Bays in eastern Long Island, NY, and other areas noted in the text. Modified from Tettelbach et al. (2015).

been used to track the success of restoration efforts with different species of scallops (Morgan et al. 1980), clams (Manzi 1990; Zarnoch et al. 2015), abalone, and lobsters (Bell et al. 2005).

The primary strategies used for restoration/enhancement of bivalve mollusk populations are (1) protection/concentration of existing stocks, (2) transplantations from more robust natural stocks, if available, or most commonly (3) plantings of hatchery-reared larvae, juveniles, or adults (Goldberg et al. 2000; Arnold 2001; Leverone et al. 2010). These approaches are sometimes followed for put-and-take fisheries, but are most often done to create spawner sanctuaries to increase the numbers/density of adult broodstock to ensure higher probabilities of fertilization success when spawning occurs (Peterson & Summerson 1992; Peterson et al. 1996; Tettelbach et al. 2011, 2013).

In our ongoing bay scallop, *Argopecten irradians irradians*, restoration program in the Peconic Bays of eastern Long Island, NY, United States (Fig. 1; Tettelbach & Smith 2009) we have planted millions of juvenile hatchery-reared scallops that serve as broodstock when they mature. Analyses of potential changes in contributory factors before and after commencement of restoration (e.g. water temperature, salinity, food levels, total fertilized egg production [TFEP]) confirmed that it is our restoration efforts that have driven order of magnitude increases

in larval recruitment, benthic populations, and fishery landings (Tettelbach et al. 2013, 2015). Initially, we planned to track the contribution of our planted broodstock by utilizing DNA markers developed for bay scallops (Seyoum et al. 2003; Roberts et al. 2005). However, this approach was abandoned after initial study revealed these markers were unable to resolve subpopulations at the scales needed for source tracking (Kretzmann et al., unpublished data).

We therefore followed an alternate strategy in an attempt to track the potential contribution of planted broodstock by rearing and out-planting large numbers of an uncommon color morph (Bell et al. 2005); in this case, we planted “skunk” bay scallops: individuals whose shells possess one or more white radial lines on a background that is usually gray to black (Fig. 2). This color morph is uncommon in New York as well as in other native bay scallop populations, with observed frequencies of 1.5% in Connecticut (Stiles et al. 2008) and $\leq 1.5\%$ in Nantucket, Massachusetts (S. Tettelbach, personal observation). Shell color in some molluscan species has been shown to be heritable, but in others it may also vary due to environmental influences (Williams 2017; Zhao et al. 2017). In bay scallops, shell color and pattern have been shown to be genetically determined (Kraeuter et al. 1984; Adamkewicz & Castagna 1988;



Figure 2. Skunk (one or more white radial lines) and nonskunk bay scallops, *Argopecten irradians irradians*, from the Peconic Bays, Long Island, NY, United States. Photo: S. Tettelbach.

Qin et al. 2007; Teng et al. 2018); this is also true for other scallop species (Wolff & Garrido 1991; Zheng et al. 2013; Zhao et al. 2017), with color likely related to the deposition of different pigments, for example, melanin (Sun et al. 2016). Through self-crosses of *A. irradians*, which are simultaneous hermaphrodites, Adamkewicz and Castagna (1988) concluded that the background shell color is determined by a single gene, with rarer yellow or orange colors being dominant to white; more diverse overlying patterns and pattern colors, which may almost completely obscure the underlying background color (Elek 1985; Elek & Adamkewicz 1990), are under control of 1 or more additional genes. While specifics of inheritance of the skunk color pattern in *A. i. irradians* have not been determined, our decision to employ this morph in the present study was based on two pertinent studies: (1) Stiles et al. (2008) showed that crosses of two skunk parents yielded $\geq 50\%$ skunk offspring and that skunks and nonskunk broodstock exhibited equivalent survival in nets placed in the field, and (2) Weinstock (2010) found no differences in skunk survival relative to “nonskunks” in laboratory studies of predation on juvenile bay scallops by scup, *Stenotomus chrysops*, the most abundant finfish predator of bay scallops in Long Island waters. However, in contrast to the findings of Stiles et al. (2008) and Weinstock (2010), after we planted high numbers of skunks, we observed from 5 to 41% higher overwinter mortality of skunks versus nonskunks for three successive years (= 3 generations: Belding 1910) and thus decided to discontinue the strategy. Here, we report 12 years of data on temporal and spatial changes in the relative frequencies of skunks in natural populations in the Peconic Bays, NY, both

before and after planting of skunk broodstock. In light of several unexpected results, we discuss the pros and cons of this approach in the context of restoration efforts.

Methods

Bay scallop broodstock (skunk and nonskunk: Fig. 2) were collected in April–May of each year (2007–2017) from natural Peconic Bay, NY, populations (Fig. 1), then conditioned and induced to spawn in the laboratory during May–June via thermal induction methods (Loosanoff & Davis 1963; Stiles et al. 2008). Offspring were grown out in intermediate culture until they were large enough (≥ 35 mm shell height, SH) to be stocked into lantern nets for overwintering at our primary spawner sanctuary in Orient Harbor, NY (Fig. 1), or planted directly to the bay bottom (“free-planted”) at 10 other locations; these served as broodstock themselves when they spawned the next year (Tettelbach & Smith 2009). The proportion of hatchery-reared skunks produced in a given year (Table 1) was usually determined at the time of stocking/planting in November–December, while the number of skunks surviving to spawn (Table 1) was estimated in April–June of the following year during restocking of lantern nets or via in situ quadrat surveys of free-planted stocks (Tettelbach & Wenczel 1993).

Proportions of skunk scallops present in natural populations were examined at three F1 life stages: postlarval settlement (i.e. postset or spat), large juvenile, and adult, for successive cohorts. Larval settlement was monitored over the full reproductive period (early June–November, 2007–2018) using spat

Table 1. Numbers and proportions of skunk bay scallops, *Argopecten irradians irradians*, of the total planted in fall 2007–2013 as part of our restoration efforts and estimated numbers surviving to spawn the following spring. Deliberate production/planting of skunks was discontinued after 2010.

Year	Total # Scallops Planted	# Skunks Planted	% Skunks Planted	Total # scallops Surviving to Spawn the Following Spring	# Skunks Surviving to Spawn the Following Spring
Fall 2007	633,400	15,138	2.39	254,776	6,089
Fall 2008	1,118,700	275,418	22.90	328,707	75,274
Fall 2009	855,000	129,533	15.15	338,560	51,292
Fall 2010	556,675	502,232	90.22	256,812	231,696
Fall 2011	524,000	0	0	186,342	0
Fall 2012	765,000	29,682	3.88	290,505	11,272
Fall 2013	674,500	12,074	1.79	413,750	7,406

collectors, plastic mesh bags into which scallop larvae will settle at the time of metamorphosis; these were placed at 24 sites throughout the Peconic Bay system (Tettelbach et al. 2013). Proportions of skunks among wild F1 juveniles and adults, respectively, were estimated from in situ collections of scallops along 50 m × 1 m belt transects at 20 sites (many being those at which spat collectors were also deployed) in fall (October–early November) or spring (March–June) (Tettelbach et al. 2015). Characterization of skunks and nonskunks was done by visually examining the dorsal shell of all individuals obtained in a given collection. This was very straightforward in large juveniles, whose shells were virtually free of epibionts. However, this was more difficult with adults, which often had extensive growth of the slipper snail, *Crepidula fornicata*, other epifauna, or algae. Shells of spat were almost always clean, but characterization of color patterns at this stage was somewhat subjective as skunk stripe patterns are not always fully evident (Miller 2017). Therefore, skunk spat were classified as those with white lines that ran more than half the distance from the umbo to the ventral margin of the shell, and/or which had more than one white line.

We previously showed that there was a strong linear correlation ($r = 0.971$) between an index of TFEP and observed larval recruitment (mean number of bay scallop spat per bag per year) resulting from spawns of adult scallops in natural and planted populations in Orient Harbor, the primary site of our restoration

efforts (Tettelbach et al. 2013). Model calculations of TFEP (Tettelbach et al. 2013) were based on in situ observations of adult scallop abundance and density (prior to spawning) at multiple sites within this embayment and estimated probabilities that spawned eggs were successfully fertilized. For this study, we examined whether our plantings of skunk broodstock led to a concomitant increase in F1 skunk frequencies ($F_{F1 \text{ skunk}}$) by calculating TFEP values for skunks ($TFEP_{\text{planted skunks}}$) relative to $TFEP_{\text{overall}}$ (wild + planted) for the scallop population in Orient Harbor (Table 2). We used the same model developed by Tettelbach et al. (2013), but made an adjustment for the percentages of F1 skunks resulting from broodstock plantings with differing skunk frequencies. We assumed 50% F1 production of the skunk phenotype from adult skunk × skunk crosses, based on findings of Stiles et al. (2008), then scaled down expected F1 skunk production, in a linear fashion, when the frequency of skunks among planted broodstock was lower than 100%. For example, for our 2008 planting, where the proportion of skunks was 0.229 (22.90%) of the planted stock, we computed the frequency of the skunk phenotype to equal 0.1145 (11.45% = 0.229 × 50%) of the F1 fertilized egg production. Next, after Tettelbach et al. (2013), we calculated:

$$TFEP_{\text{planted skunks}} = \# \text{surviving skunk broodstock} \\ \times 0.5 \times 1 \times 2 \times 10^6 \text{ eggs produced}$$

Table 2. Expected versus observed changes in F1 skunk frequencies ($F_{F1 \text{ skunk}}$) among spat and large juveniles (lg juv.) in Peconic Bay scallop, *Argopecten irradians irradians*, populations following planting of skunk broodstock in Orient Harbor, NY, the site of the majority (but not all) of our restoration efforts (Tettelbach et al. 2013, 2015). Expected increases in $F_{F1 \text{ skunk}}$ were calculated by examining the contribution of TFEP (Tettelbach et al. 2013) from spawns of planted skunks relative to the overall population (see Methods for details). Deliberate production/planting of skunks was discontinued after 2010.

F1 Cohort (x)	F_{skunk} Broodstock Cohort (x - 1)	TFEP (planted skunks) (10^9)	TFEP (total) (10^9)	Expected Increase in $F_{F1 \text{ skunk}}$ From Skunk Plantings	Total Expected $F_{F1 \text{ skunk}}$ (natural + planted)	Observed $F_{F1 \text{ skunk}}$ (spat)	Observed / Expected $F_{F1 \text{ skunk}}$ (spat) (%)	Observed $F_{F1 \text{ skunk}}$ (lg juv)	Observed/ Expected $F_{F1 \text{ skunk}}$ (lg juv) (%)
2007	0.036	2.151	201.641	0.0107	0.0467	0.058	124.2	0.044	94.2
2008	0.026	57.513	1,398.547	0.0411	0.0671	0.043	64.1	0.047	70.0
2009	0.041	38.688	1,183.283	0.0327	0.0737	0.060	81.4	0.064	86.8
2010	0.043	226.632	3,268.006	0.0693	0.1123	0.048	42.7	0.062	55.2
2011	0.036	0	3,805.643	0	0.0360	0.039	108.3	0.044	122.2
2012	0.032	6.227	8,626.996	0.0007	0.0327	0.049	149.8	0.042	128.4
2013	0.037	4.206	8,913.046	0.0005	0.0375	0.048	128.0	0.057	152.0

where # surviving skunk broodstock = total broodstock scallops surviving $\times F_{\text{planted skunks}}$; 0.5 is the proportion of skunks produced from skunk \times skunk crosses; 1 is the rate of fertilization success at high planting densities; and 2×10^6 eggs produced per scallop is the number from Belding (1910) (Table 2). We assumed that survival of skunk and nonskunk broodstock was the same (Stiles et al. 2008). We believe that the skunk allele is probably recessive, given the low frequency of skunks in natural populations; so, to simplify matters here, we conservatively assumed 0% production of skunks from skunk \times nonskunk crosses. Expected changes in F1 skunk frequencies ($F_{\text{F1 skunk}}$) among spat and large juveniles in successive years were computed for the 4 years of deliberate skunk plantings (2007–2010) and for 3 years after these were discontinued (2011–2013) (Table 2). These were then compared to observed $F_{\text{F1 skunk}}$ values.

Data were also opportunistically collected on the relative survival of skunk versus nonskunk postset (≥ 1 mm SH) in small (10 cm \times 20 cm) artificial seagrass units (ASUs; Virnstein & Curran 1986; Bologna & Heck 2000), designed to mimic eelgrass, deployed at one of our spat collection sites (41.138119°N, 72.263322°W) in Hallock Bay, NY (Fig. 1), during summer 2010. These data reported here represent part of a series of studies on scallop settlement, growth, and survival (Carroll 2012). Briefly, 10 ASUs were deployed at the Hallock Bay site—6 small (8.5 m²) and 4 large (17 m²), constructed by tying green polypropylene ribbon to a Vexar mesh at a shoot density (500 shoots m⁻²), blade width (5 mm), blade number (4), and canopy height (35 cm) within the range of naturally occurring eelgrass beds in eastern Long Island (Pohle et al. 1991). A series of small (10 \times 20 cm) ASUs with 10 shoots were attached to larger units deployed at this site to serve as recruitment collectors. Five shoots were enclosed in an 800 μ m mesh bag, large enough to allow scallop larvae to enter and metamorphose, but small enough to prevent entry by potential predators. The other five shoots were left exposed. This design allowed us to estimate postset survival by comparing the number of individuals outside the bag (recruits) to the number inside the bags (settlers; Carroll et al. 2012). Although that study was designed to quantify edge effects, due to low overall numbers of skunk scallops all collectors across all ASUs were pooled for analysis.

Statistical analyses focused on examining differences in skunk frequencies at different life history stages. A Spearman rank correlation matrix was used to determine whether the numbers of skunk spat, large juvenile, and adult scallops in natural populations for given year classes (cohorts) were correlated to each other and/or to the numbers of planted skunk broodstock that produced these respective cohorts. The broodstock-offspring relationships were considered the best way to test the hypothesis that skunk plantings could be used to track the contribution of our restoration efforts to natural population growth. As field observations suggested differential overwinter survival (proportional change in prevalence of large juveniles in fall to adults the following spring) of skunks and nonskunks, for several successive cohorts, we used a sign test for two-sample paired data to test whether patterns differed for the two groups. As there was considerable interannual variability in

overall numbers of scallops as well as skunk frequencies in natural populations at the level of sampling location from 2007 to 2018, which might have obscured overall patterns (Tettelbach et al. 2013, 2015), we focused on examining skunk frequencies at different life stages for respective cohorts for all sites combined. For the ASU data, we sought to answer two questions that would offer support to our hypothesis about differential skunk mortality. First, we examined whether skunk proportions in scallop set (inside collectors) differed from the skunk proportion in the recruits (outside collectors; Carroll et al. 2012). Second, we were interested in estimating the survival of skunk scallops relative to nonskunk scallops. Since the overall number of scallops in ASUs exhibiting the skunk pattern was low ($n = 219$: $\sim 11\%$ of the total number of postset), data from all collectors were pooled for each of the three respective sampling dates. Since the sample size was too small ($n = 3$) to determine whether parametric assumptions were met with our proportion data, we ran two Mann–Whitney U tests with an $\alpha = 0.05$: one to determine if skunk proportion was higher in settlement versus recruitment collectors and another to compare survival between the two groups. Due to the small sample size, results were interpreted cautiously.

Results

In natural populations, prior to planting of skunks, overall frequencies of large juvenile and adult skunks in the 2006 cohort were 0.038 and 0.036, respectively (Fig. 3). Skunk proportions among F1 spat, large juveniles, and adults, after we began planting skunks (2007–2017 cohorts), ranged from 0.039 to 0.061, 0.028 to 0.064, and 0.006 to 0.055, respectively (Fig. 3). When year-to-year skunk frequencies among F1 spat, large juvenile, and adult scallops were compared to numbers of planted skunk broodstock surviving to spawn (Fig. 3), there appeared to be an increase in skunk frequencies in the F1 large juvenile stage right after high numbers of skunk broodstock were planted in 2008–2010. Respective increases in skunk frequencies for these three cohorts were 23, 68, and 63%, compared to preskunk planting baseline levels from fall 2007. Also, there appeared to be a sharp drop (30%) in skunk frequencies among F1 large juveniles from 2011 to 2012, right after we discontinued plantings of skunk broodstock. For years in which skunks were planted, TFEP calculations revealed that expected increases in $F_{\text{F1 skunk}}$ ranged from 0.0005 (2013) to 0.0693 (2010) (Table 2). Observed $F_{\text{F1 skunk}}$ levels for spat and large juveniles were sometimes lower ($<100\%$) and sometimes higher ($>100\%$) than expected increases in $F_{\text{F1 skunk}}$ (Table 2). Interestingly, during the years in which we planted the highest frequencies and numbers of skunk broodstock (2008–2010), observed levels were always lower than expected; the converse was true for years in which fewer skunks were planted (2007, 2011–2013).

The Spearman rank correlation matrix (Fig. 4) revealed that when data for the 12 years were examined there was no significant correlation between the numbers of planted skunk broodstock and the frequency of F1 skunks in natural populations—for spat, large juveniles, or adult scallops. Furthermore, when assessed as cohorts, there were no temporal

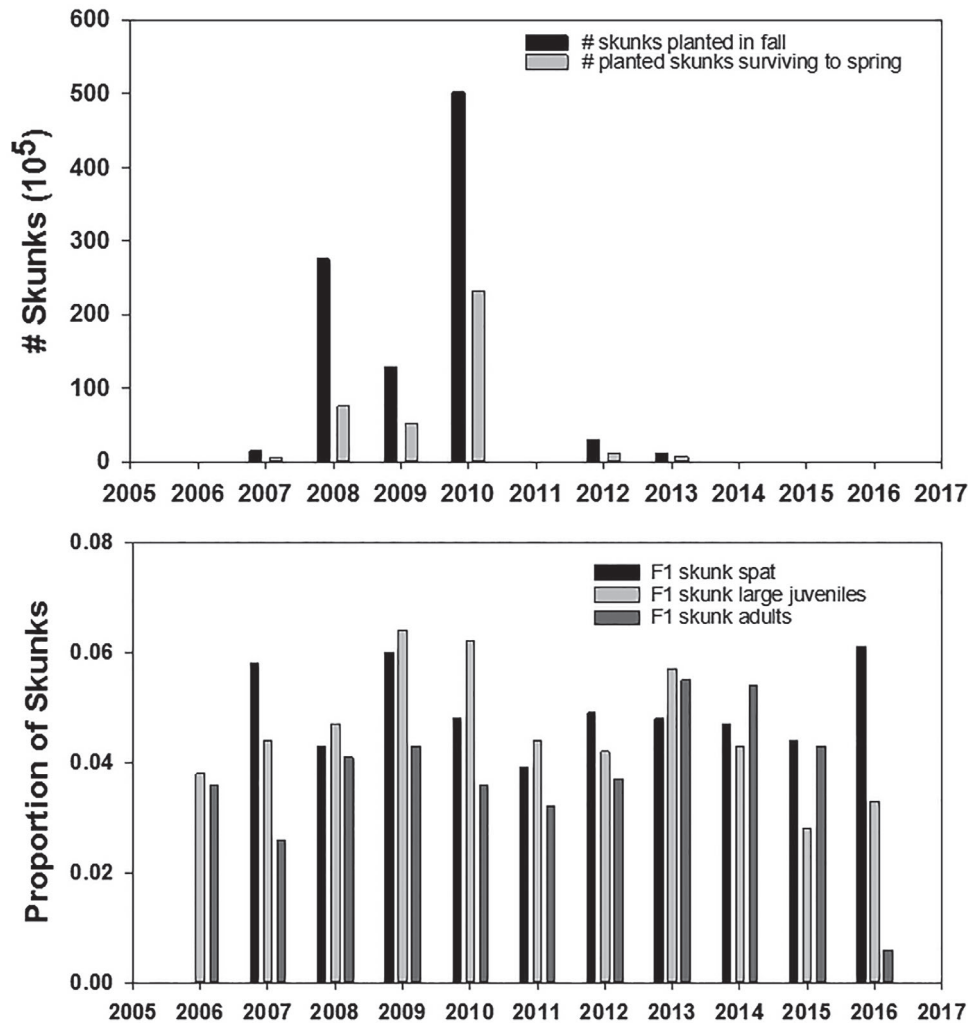


Figure 3. (A) Numbers of skunk bay scallop broodstock planted in fall and respective numbers of these surviving to the next spring (when they spawned), 2006–2017; 0 skunks were planted in 2011, no data were available for 2006 and 2014–2016. (B) Proportions of skunks among different F1 life stages: spat, large juveniles, adults. F1 life stages in (B) are aligned directly below the respective broodstock cohorts in (A); that is, no lag time is shown in the plots.

trends in these relationships (Fig. 4). In fact, the only significant correlation of skunk frequencies between scallop life history stages was between the number of skunks planted in fall and those censused the following spring ($r = 0.9755$, $p < 0.01$; Fig. 4). When the Spearman rank correlation matrix included only the 2007–2013 data (we did not quantify skunk frequencies among our broodstock after 2013, although we know they were low), we obtained very similar correlation coefficients and patterns of statistical significance as those yielded by the larger dataset.

Overwinter survival of large juvenile skunks in natural populations was lower than that of nonskunks for eight consecutive years (winters of 2007–2008 through 2014–2015) (Fig. 5). During the next two winters, skunks exhibited higher survival than nonskunks, before again showing differentially higher mortality in 2017–2018 (Fig. 5). A sign test showed that median proportional overwinter loss of skunks (0.55) was higher than that of nonskunks (0.46; $S = 8$, number of differences = 10,

$p = 0.055$). The significant ($p < 0.01$) correlation between numbers of skunks planted in fall and those found in the spring strengthens our assertion that skunks suffered differentially higher overwinter mortality.

In the 348 small ASUs deployed in Hallock Bay in 2010, across three collection periods, a total of 1,968 postset scallops were collected, of which 219 (~11%) were skunks. The majority of these postset were found within the bags (1,889 total settlers, including 214 skunks), while the number outside bags (79 total recruits, including 5 skunks) was much lower. After pooling data for respective sampling dates, the calculated average proportion of skunk settlers (0.107 ± 0.009) was found to be double that of recruits (0.050 ± 0.025 ; $t = 2.13$, $p = 0.06$; Fig. 6A). Temporal survival rates of settled scallop larvae were highly variable: total overall survival was ~10–11% in the 15 July and 26 August collections, but only ~2% in the 5 August collection. Estimated survival of nonskunk scallops ($9.9 \pm 4.0\%$) was 3.5 \times greater than survival of skunk scallops ($2.8 \pm 2.2\%$), but due

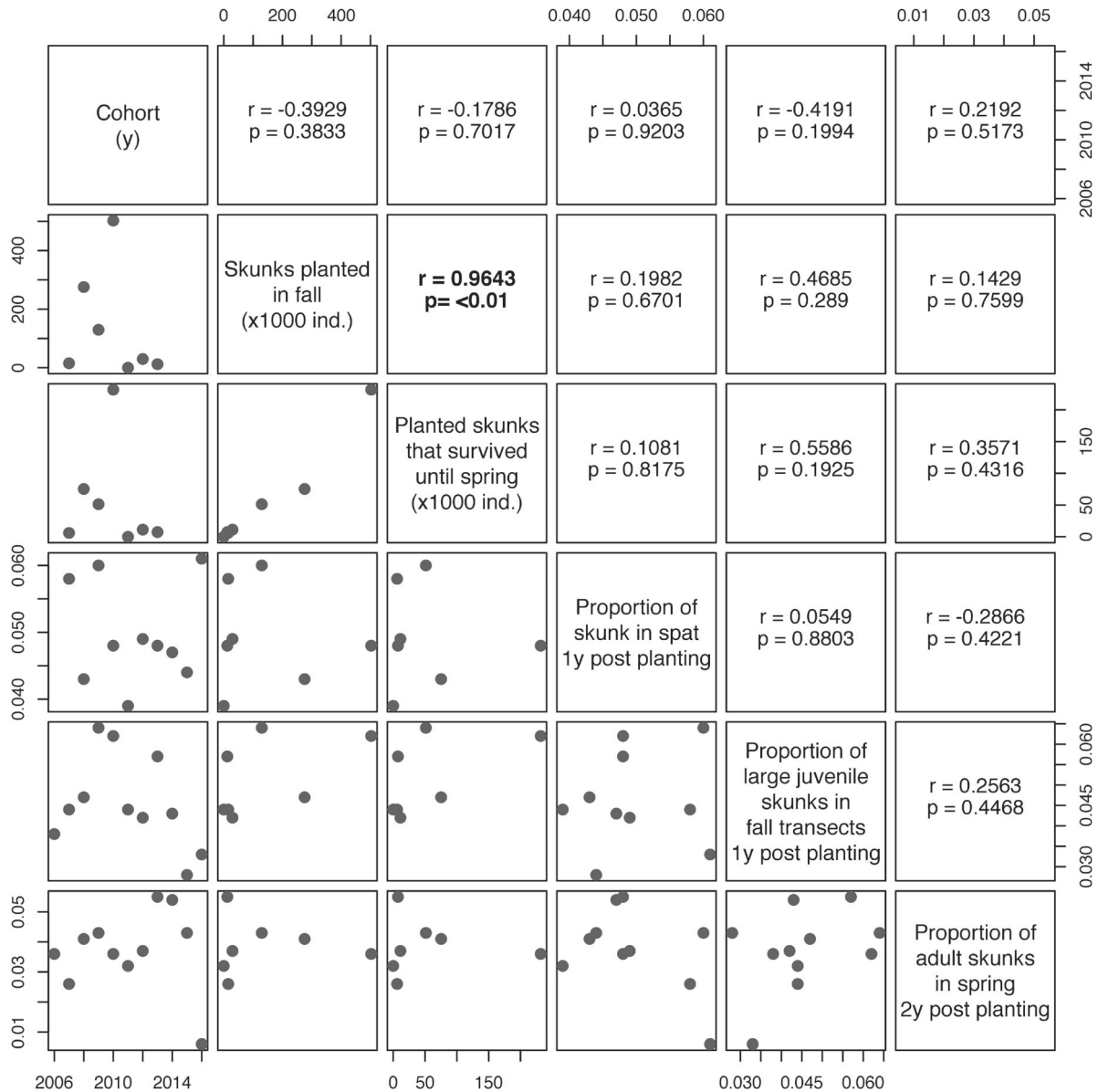


Figure 4. Spearman rank correlation matrix used to examine correspondence of skunk color morph frequencies at different life stages for respective cohorts (year classes) of bay scallops in the Peconic Bays, NY, United States: (large juvenile) skunks planted in fall (numbers), planted skunks that survived until spring (=broodstock [numbers]), and proportions of skunks among F1 spat (small juveniles), large juveniles, and adults. The “cohort” block was used to examine whether temporal patterns of correlations varied significantly between different scallop cohorts.

to the temporally variable survival rates, this was marginally nonsignificant ($t = -1.499$, $p = 0.11$; Fig. 6B).

Discussion

Estimating the contribution of hatchery-raised broodstock to changes in wild adult populations is notoriously difficult, contingent upon stable identifiable markers that pass predictably to offspring and on effective monitoring programs (Bravington & Ward 2004; Bell et al. 2005). Yet, the value of assessing the role of active restoration in supporting imperiled populations and species can be worth the added effort. Large restoration

programs are costly and determining success is critical to public and commercial stakeholder buy-in as well as maintaining funding levels for requisite periods of time (Arnold 2001; Schulte et al. 2009; Tettelbach et al. 2015). In this article, we report on our attempt to use an uncommon “skunk” color morph for tracking hatchery contributions to gains in wild bay scallop populations. Increases in skunk frequencies in Peconic Bay populations in the 3 years right after we planted large numbers of skunk broodstock initially suggested our approach was successful. However, observed skunk frequency levels in the F1 life stages ($F_{F1 \text{ skunk}}$) did not match well to levels expected after skunk broodstock plantings and correlations

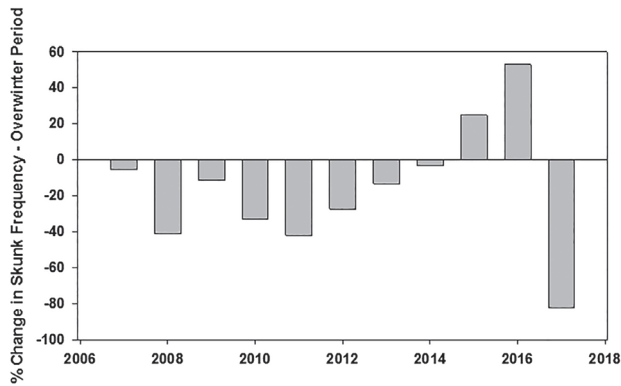


Figure 5. Overwinter survival (October/early November to following spring) for different cohorts of wild large juvenile skunk bay scallops; data pooled for all study sites where population surveys (Tettelbach et al. 2015) were conducted in the Peconic Bays, NY, United States. Bars above and below the horizontal line signify that the frequency of skunks increased or decreased, respectively, from fall to spring.

between skunk frequencies at different life stages were low. Unfortunately, the expected skunk signal was not maintained during the balance of a 12-year period, despite clear evidence that our extensive restoration efforts were driving the dramatic increases in Peconic Bay scallop larval recruitment, benthic population sizes, and fishery landings (Tettelbach et al. 2013, 2015). Other studies have directly tracked the success of planting hatchery-reared bivalve mollusks by examining survival of uncommon to rare color morphs, including the *notata* variety of *Mercenaria mercenaria* (Zarnoch et al. 2015), yellow to orange *Argopecten purpuratus* (Wolff & Garrido 1991), and orange *Argopecten irradians irradians* (Morgan et al. 1980). However, our attempt to track the contribution of planted populations to the F1 generation by looking for changes in phenotypic, rather than allelic, frequencies (Bell et al. 2005) ultimately proved to be unsuccessful. Nevertheless, this is valuable to know as it means this approach may be too blunt to use in the context of restoration efforts.

More specific markers (e.g. mtDNA and SSRs) have been used effectively to track bay scallop restoration (Seyoum et al. 2003; Roberts et al. 2005) and gene flow between populations (Marko & Barr 2007), but their utility depends on detecting sufficient differences between broodstock and hatchery-reared offspring versus animals from natural populations (Bell et al. 2005). We used the same markers as those developed by Seyoum et al. (2003), but only saw limited genetic differences in Long Island bay scallops sampled from populations throughout the Peconic Bays and from geographically distant Great South Bay (Kretzmann et al., unpublished data). We did not have the resources to develop new markers that might be more specific to Long Island populations and thus resorted to the strategy of employing the skunk color morph.

There are several possible reasons why the skunk signal did not work well over the long term. Year-to-year variability in skunk frequencies within the natural population, for which we do not have a good estimate, may simply be too high to detect the skunk signal at the level of expected increases in $F_{F1 \text{ skunk}}$. While

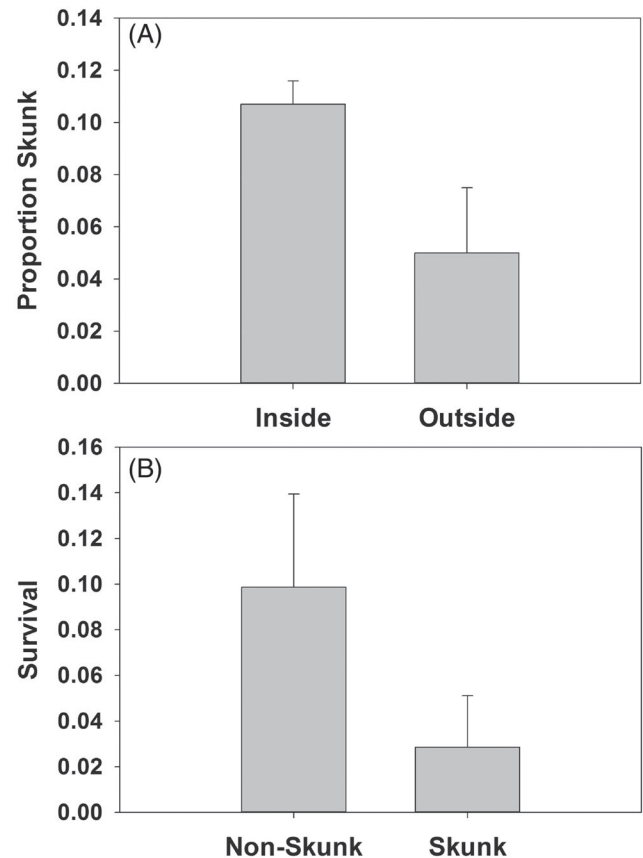


Figure 6. (A) Mean proportion of skunks (+1 SE) among all postset bay scallops found on shoots inside and outside mesh bags in small (10 cm \times 20 cm) ASUs deployed in Hallock Bay, NY, United States, in summer 2010: $t = 2.130$, $p = 0.06$. (B) Mean proportional survival (+1 SE) of skunk scallops in these same ASUs: $t = -1.499$, $p = 0.11$.

Stiles et al. (2008) showed that skunk \times skunk crosses in the laboratory yielded $\geq 50\%$ skunk F1 offspring, we do not know if this production ratio is the same under field conditions, nor do we know the frequencies of skunk offspring that result from skunk \times nonskunk crosses. All of these questions apply to skunk broodstock in planted as well as natural populations. While we have shown that planted hatchery-reared scallops spawn at the same time as those from natural populations (Tettelbach et al. 2002), it is unknown whether there might be differences in the timing of reproductive development/spawning among different color morphs. The apparent signal of higher skunk frequencies (although lower than expected) in the first 3 years after planting large numbers of skunk broodstock, and its eventual loss over time, may reflect the relatively higher contribution of our planted stocks to overall fertilized egg production in the first few years after we commenced our restoration efforts and a dilution of their overall contribution as natural populations expanded during subsequent years (Tettelbach et al. 2013, 2015).

Another possible explanation for the poor correlation between numbers of planted skunk broodstock and frequencies of the skunk phenotype among offspring may be related to changes in the appearance/number of white stripes (including

their loss) on bay scallop shells, particularly in the small juvenile stage (Miller 2017). In a group of small (5–21 mm SH) juvenile skunks, which were transplanted from spat collectors to suspended nets in Orient Harbor for ~5 months (July to December 2013), stripe numbers were seen to decrease (i.e. laying down of stripes was discontinued) in 65 of 480 (13.5%) of the individuals (Miller 2017). However, larger juveniles with a stripe running a short distance from the umbo, but which is not continued on the rest of the shell, are only seen uncommonly (probably in less than 3% of individuals) in wild, benthic Peconic populations (S. Tettelbach, personal observation). Therefore, we suggest that changes in stripe number, and potential misclassifications of the skunk phenotype, are not likely to explain the poor correlations between skunk frequencies at different life stages.

A more likely reason for the poor correlations between skunk frequencies at different scallop life stages is the lower survival of skunks in the period soon after larval settlement as well as during the overwintering of large juveniles. Even though our statistical analyses showed marginally nonsignificant differences in mortality of skunks and nonskunks at these two life stages, the fact that we saw higher overwinter mortality of skunks in 9 of 11 years is intriguing. The pattern may also manifest at intermediate juvenile sizes, although these were not examined in this study. Further work on fine-scale habitat utilization may resolve this issue.

Overwinter mortality of juvenile bay scallops due to passive burial by shifting sediments is an important source of mortality in planted and natural populations (Tettelbach et al. 1990), but it is difficult to imagine that skunks are more susceptible to this process than nonskunks. If skunks do suffer reduced fitness a more plausible cause would be differential predation pressure. Although Weinstock (2010) found survival of 10–12 mm SH juvenile skunks to be equivalent to nonskunks in laboratory predation experiments with scup, *Stenotomus chrysops*, the relative survival of the two color morphs may differ depending on the visual capabilities of different species of predators and the type of substrates on which they are found (e.g. Palma & Steneck 2001). Another explanation for higher predatory mortality is that skunks have weaker shells, making them more vulnerable to crushing by finfish and crustacean predators (Miller 2017). Even though the species/sizes of predators that prey upon postset bivalves (Gosselin & Qian 1997; Newell et al. 2000; Kraeuter 2001) and large juveniles (Belding 1910; Tettelbach 1986; Peterson et al. 1989) are very different, either or both of the above characteristics of skunks may make them more susceptible to predation by both visual as well as chemosensory feeders. It should be noted that the critical overwinter period, as considered here, occurs between our fall (October–early November) and spring (usually May–June) population surveys. Most predators of *A. irradians irradians* are inactive during January and February (Tettelbach 1986), other than sea stars (*Asterias forbesi*: Auster & DeGoursey 1994), but sea stars are now rare in most parts of the Peconic Bays (Tettelbach et al. 2015). Thus, if due to predation, differential overwinter mortality of skunks is more likely to occur during the warmer months of this period (Tettelbach 1986).

Another potential cause of differential overwinter mortality, which has been investigated in the common white and uncommon *notata* color morphs of the hard clam *Mercenaria mercenaria*, is related to physiological tolerances. Zarnoch and Sclafani (2010) found that *notata* clams exhibited poorer survival than white clams, possibly due to differences in phospholipid to sterol ratios that make the *notata* morph less resistant to cold stress (Pernet et al. 2006). Physiological differences between bay scallop color morphs are unknown, but could represent a fruitful area of research.

The consistently higher overwinter mortality of skunks versus nonskunks in the 3 years after we began planting high numbers of skunk broodstock is what prompted us to discontinue their use as markers. This decision was supported by higher differential mortality over 8 consecutive, and in 9 of 11, years. We can only speculate why skunk survival was higher during the winters of 2015–2016 and 2016–2017, but despite the ancillary benefits of planting skunks (see below), the decision to discontinue our strategy was not a difficult one. Poor survival of planted orange color morphs of *A. irradians irradians* (Morgan et al. 1980) and yellow *A. purpuratus* (Wolff & Garrido 1991) led to similar decisions. In general, genetic tags may be less likely to endure differential mortality than uncommon phenotypes (see Bell et al. 2005). In our bay scallop restoration efforts, the goal is to produce as many broodstock as is logistically possible in order to maximize the production of larvae that can disperse and settle to reestablish/bolster natural populations (Tettelbach et al. 2013). Although the survival of skunks in nets is expected to be the same as that of nonskunks (Stiles et al. 2008), fewer surviving recruits translates to lower population sizes and on-bottom densities, which in turn means lower numbers of broodstock, lower fertilization success of spawned eggs (Peterson et al. 1996; Tettelbach et al. 2013), and a reduced capacity for population rebuilding. We would recommend that other restoration programs evaluate the relative performance/survival of alternate color morphs before beginning to produce them and, of course, to ensure that hatchery-reared stocks are not genetically inferior to genotypes present in natural populations (Caddy & DeFeo 2003; Zohar et al. 2008).

In a surprising twist, harvested skunk scallops were regarded by many local commercial fishermen as “our” scallops even though we did not widely publicize that we were planting skunks (N. Andruski 2012, Bayman, Southold, NY). Thus, an important benefit of our strategy was that it helped elevate the awareness and positive perception of our restoration program among the fishing community. This, along with continued growth of Peconic Bay scallop population sizes and a return of sustainable commercial harvests (Tettelbach et al. 2015; New York State Department of Environmental Conservation 2019), has translated to more of the public and commercial stakeholders offering support for our ongoing restoration efforts.

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