Forecasting the Genetic Impacts of Net Pen Failures on Gulf of Mexico Cobia Populations Using Individual-Based Model Simulations

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Abstract

Offshore net pen fish farming provides a cost-efficient means for production of marine finfish, and there is great interest in development of net pen operations in domestic waters. However, there are concerns over the possible genetic and ecological impacts that escaped fish may have on wild populations. We used individual-based simulations, with parameter values informed by life history and genetic data, to investigate the short-term (50 years) impacts of net pen failures on the genetic composition of cobia, Rachycentron canadum, stocks in the Gulf of Mexico. Higher net pen failure rates resulted in greater genetic impacts on the wild population. Additionally, the use of more genetically differentiated source populations led to larger influxes of non-native alleles and greater temporal genetic change in the population as a result of net pen failure. Our results highlight the importance of considering the appropriate source population for broodstock collection in net pen aquaculture systems, and help to provide a general set of best management practices for broodstock selection and maintenance in net pen aquaculture operations. A thorough understanding of the genetic diversity, stock structure, and population demography of target species is important to determine the impact escapees can have on wild populations.

Global aquaculture production has expanded rapidly over the last half-century, with present production levels accounting for more than 40% of the global seafood supply (FAO 2014). Simultaneously, there is growing interest around the world in the use of offshore net pen fish farms for grow-out (Bostock et al. 2010). In the U.S., net pen aquaculture has lagged behind other countries, with the majority of commercial net pen facilities producing Atlantic salmon (Rust et al. 2014). However, this is likely to change due to the increased cost efficiency and production yield attainable in net pen aquaculture. For example, specific growth rates of greater than 2% per day are possible using this technology, resulting in impressive gains in mass during grow-out (Benetti et al. 2010). The expansion of net pen farming has raised concerns over the possible genetic and ecological impacts that escaped fish may have on wild populations. Fish can escape from net pen facilities for a variety of reasons including holes in the net that develop from normal wear and tear, loss during transfer, grading, or harvest, and through catastrophic pen failures (e.g., during severe storms). Large escapes from offshore net pen facilities have led to calls for stricter industry regulations (e.g., Esmark et al. 2005); however, recent advances in industry practices have helped to reduce the magnitude of many of the potential ecological impacts (Rust et al. 2014). Nonetheless, there are still concerns over the influences escaped fish might have on genetic diversity. For instance, large-scale escapes from net pen holding facilities could lead to a variety of genetic impacts on wild populations, including reductions in effective population size ($\underline{N}_{\underline{e}}$; Ryman and Laikre 1991), swamping of the locally-adapted gene pool, and/or alterations in natural patterns of population structure (Laikre et al. 2010). A proactive approach for evaluating and minimizing the potential negative genetic influences of escaped farmed fish would provide a valuable tool for resource managers.

Conceptually, large-scale escapes of cultured fish from net pen fish farms share some similarities with hatchery-based population supplementation and stock enhancement. While intentional and accidental releases of cultured fish apply similar pressures on the genetic diversity of the wild population, the management decisions made in the hatchery (which ultimately influence the magnitude of genetic impacts) will likely differ in these situations because of the respective goals of stock enhancement and commercial-scale aquaculture. For instance, stock enhancement programs would be less likely to use domesticated broodstock and would place a higher priority on maximizing the number of broodstock in the hatchery. Conversely, production and yield are more important considerations in commercial-scale aquaculture programs. As net pen escapement represents an extreme case of stock enhancement (i.e., small numbers of potentially artificially selected broodstock, possibility of using non-native broodstock sources, etc.), the risks to native genetic diversity should be thoroughly investigated prior to the expansion of offshore net pen aquaculture.

One promising candidate for net pen aquaculture is cobia, Rachycentron canadum (Linnaeus, 1766), a large, pelagic, migratory species with a nearly cosmopolitan distribution throughout tropical and subtropical waters. The species is highly prized in recreational fisheries and important in aquaculture. Within the U.S., cobia occupies a range throughout the South Atlantic and Gulf of Mexico, and is regulated via the South Atlantic Fishery Management Council (SAFMC) and the Gulf of Mexico Fishery Management Council (GMFMC). Cobia are batch spawners, capable of spawning multiple times within a season (Biesiot et al. 1994; Lotz et al. 1996). Spawning occurs from April through September in the wild (Lotz et al. 1996; Smith 1996; Burns et al. 1998; Brown-Peterson et al. 2001), with regional peaks in spawning correlating with the annual cobia migration from Florida to Massachusetts. Peak spawning in South Carolina occurs in May (Shaffer and Nakamura 1989; Burns et al. 1998) with spawning peaks in North Carolina during June (Smith 1996) and Chesapeake Bay in June and July (Joseph et al. 1964). Annual inshore aggregations in certain high-salinity estuaries along the Atlantic and Gulf coasts are hypothesized to be associated with feeding or reproduction (Joseph et al. 1964; Richards 1967; Hassler and Rainville 1975; Lotz et al. 1996; Smith 1996; Burns et al. 1998), and recent research has documented the presence of eggs, newly hatched larvae, and reproductively mature females within Port Royal Sound, SC verifying that fish are spawning in this inshore aggregation (Lefebvre and Denson 2012).

Recreational cobia harvests in the U.S. have been irregular over time, but generally have been on the rise, especially in estuaries where adult cobia aggregate (SC, VA), making them susceptible to over-fishing during a critical life stage. In these locations, tournaments focused solely on cobia (McGlade 2007) are common and ‘catch and release’ is the exception rather than the rule. Therefore, with continued growth in coastal human populations and increasing fishing pressure in both inshore and offshore habitats, there is an urgent need for additional fishery management tools and aquaculture production options for cobia stock enhancement. The species is a good candidate for net pen aquaculture, due to its rapid growth and high market value, and there is growing interest in developing offshore facilities for commercial-scale production

(Bostock et al. 2010). In the last 10 years, cobia aquaculture in the U.S. has moved from research and development phases to the point where some companies are now interested in deploying offshore cages for grow out.

The potential adverse impacts of net pen escapees on native populations (e.g., Naylor et al. 2005; Jensen et al. 2010) and the need for species- and life stage-specific mitigation plans, possibly requiring intensive efforts directed towards recapturing escaped fish (Skilbrei and Jørgensen 2010; Chittenden et al. 2011; Serra-Llinares et al. 2013), provide powerful motivation for the development of best management practice guidelines for the industry. Simulation models are an attractive means for short-term impact assessment and can proactively provide recommendations for responsible net pen aquaculture. Specifically, life history information and patterns of genetic variation allow the parameterization of detailed, individual-based simulation models, and outputs of these simulations can inform hatchery management practices (e.g., by providing recommendations on broodstock number and source). Model-based approaches also allow for sensitivity analyses to determine the impacts of parameter uncertainty on results. Several previous studies have used theoretical relationships (Ryman and Laikre 1991; Tringali and Leber 1999; Lynch and O'Hely 2001; Wang and Ryman 2001; Duchesne and Bernatchez 2002) or simulation models (Waples and Do 1994; Oota and Matsuishi 2005; Volk et al. 2015) to examine the impacts of cultured fish on wild population genetic diversity and fitness. Additionally, models incorporating empirical data would be a valuable tool for the adaptive management of net pen aquaculture on ecological and management time scales.

Here, we present results from a project using individual-based simulations to study the genetic impacts of cobia escapees from net pen aquaculture facilities in the Gulf of Mexico. To examine these effects, we integrated data on spawning seasonality, life history, and global population genetic structure into an individual-based simulation model. The model was then used to investigate the potential short-term (50 years) genetic impacts of net pen failures with varying magnitudes of escape, broodstock population sizes, and broodstock sources, as these are the primary adjustable variables that determine the magnitude of impacts on the wild population. The simulations used in this study were designed to provide guidance and recommendations for the responsible operation of net pen aquaculture facilities. Because the simulations are tuned to match the life history and population genetics of cobia in the Gulf of Mexico, they provide quantitative predictions on the magnitude of genetic impacts associated with net pen failure. Our results highlight the importance of considering the appropriate source for broodstock in net pen aquaculture operations, quantify levels of introgression and genetic differentiation associated with alternative management practices, and provide critical recommendations for broodstock selection and maintenance for cobia aquaculture.

Materials and Methods

Model Structure

The simulation model for our project was coded in the R statistical computing environment (R Core Team 2014) using the ‘rmetasim’ package (Strand 2002), which has been subject to extensive independent testing for reproducibility and accuracy of demography and Mendelian inheritance (Martien et al. 2009). Because of the high computational demands of the simulations, the model focused on short-term (50 years) effects of net pen failures (and resulting escapes) on standing genetic variation in native cobia stocks in the Gulf of Mexico. We chose to develop our model using an individual-based modeling approach wherein each fish in the system, including broodstock in hatcheries, fish farmed in net pens, and members of the wild stock, is modeled in silico. Individual-based models are capable of accommodating diverse data on life history, population genetics, and demographic trends. These models are also able to include stochastic processes in the simulated populations while relying on fewer assumptions (e.g., discrete generations, large population size) than coalescent or stage-based simulations. The demographic portions of our model are similar to commonly-used age-structured matrix models (Leslie 1945; Lefkovitch 1965). The expected number of individuals in each age class is determined by the equation,

$$\boldsymbol{n_{t+1}} = \boldsymbol{L n_t}$$

Where $\boldsymbol{n_t}$ is the vector of age-specific abundances and $\boldsymbol{L}$ is the Leslie matrix of age-specific fecundity and survival. Stochasticity in births and deaths in the model lead to variance around these expectations. The simulation model used in our study was developed in discrete-time, with a one-year time interval. Within each year, three events occurred: reproduction, survival, and population regulation.

To limit the size of the simulation in computer memory, early life-stages (fertilized egg production) were bypassed and the products of reproduction were young of the year (YOY) fish. By omitting this initial year of life, we effectively reduced the total number of juvenile fish simulated each year by 94%, making intensive individual-based simulation of the population tractable. Even with this simplification and the associated reduction in the number of individuals modeled in our simulations, each replicate still required ~45 minutes of computing time to complete.

The number of offspring produced by each individual female was assumed to follow a Poisson distribution with relative age-specific reproductive rates based on size (body mass, in kg) of females (Table 1). At reproduction, meiosis was simulated in both fathers and mothers, alleles from each parent were combined to form each offspring's genotype, and a mutation rate of 10^{-4} mutations per meiosis was incorporated. The simulation model assumed a strict step-wise mutation model (Ohta and Kimura 1973) for simulated microsatellite loci. Each multilocus genotype consisted of 10 unlinked microsatellite loci, with starting allele frequencies set to match empirical genetic data collected by our lab (SCDNR, unpublished data).

Survival of each individual was determined by age-specific survival rates based on the most recent stock assessment for the Gulf of Mexico cobia population (Table 1; SEDAR 2013). For each individual, a random number was drawn from a uniform distribution on a zero to one interval and evaluated to determine survival. If the random number was less than or equal to the mortality rate for individuals of that age, that particular individual was removed from the simulation. The native cobia population was modeled as three subpopulations with free migration and no genetic structure within the Gulf of Mexico. Carrying capacity was set at 130,000 fish for each subpopulation, thus the total population size in the Gulf of Mexico was 390,000 cobia. This carrying capacity was set based on estimated total biomass in the Gulf of Mexico (SEDAR 2013). In each year of the simulation, if the simulated Gulf of Mexico population size exceeded a total of 390,000 individuals, population regulation was implemented by randomly removing individuals, regardless of demographic stage, until the population size fell below carrying capacity. Thus, our model assumes a stable population of 390,000 cobia in the Gulf of Mexico throughout the simulation. Random removal of individuals ensured that the population stayed below the carrying capacity over the course of the simulations, but did not result in true density-dependence of vital rates.

We modeled the failure of net pens by simulating movement of YOY fish from hatcheries directly into native stocks (Fig. 1). Similarly, new randomly-selected broodstock individuals were rotated into the hatchery from their respective source population each year. Thus the population of cobia in the hatchery is newly formed from an initial sample of broodstock during each simulation year. Because cobia are grown in net pens during their first year, these pens were considered temporary housing of YOY fish before they are released in the event of a net pen failure, thereby providing a mechanism of simplified model construction without compromising biological realism. As fish reared in net pens are likely to be better provisioned and larger than their wild counterparts at a given age, we did not impose early-life mortality on YOY hatchery fish escaping from net pens. The majority of our simulations consider this scenario, which provides a more conservative (from a management perspective) assessment of the genetic impacts of net pen failure. However, to assess the influences of this assumption, we also simulated a smaller number of parameter combinations and replicates under an alternative model where fish were subjected to the normal early-life mortality associated with YOY fish in the wild (~94% mortality; high mortality). To facilitate interpretation of our simulation results, we focus on simulations with zero mortality of escaped fish in the first year of their lives as they provide a more direct link between the genetic impacts seen in the population and the number of fish that escape from net pens.

The initial age structure for the native Gulf of Mexico cobia stock was based on an estimate of the equilibrium age distribution derived from eigensystem analysis of the life table. Initial allele frequencies and our assumption of no population genetic structure in the Gulf of Mexico were based on data from genetic analysis of samples from across the geographic range of cobia (SCDNR, unpublished data). Similarly, initial allele frequencies in the broodstock were determined based on the allele frequencies in the source population (e.g., Gulf of Mexico, Australia, Thailand) from which they were selected.

The model was designed to simulate changes in three parameters to examine their effects on standing genetic variation in the Gulf of Mexico. The rate of net pen failure (f), the number of broodstock fish held in the hatchery ($\underline{N}_{\underline{brood}}$), and the source of broodstock (S) used to produce cobia in net pens were each varied in our simulations. Our simulation study adopted a factorial design where all possible combinations of parameter values (four values of f, four values of $\underline{N}_{\underline{brood}}$, and eight different S populations; Table 2) were simulated, resulting in a total of 128 parameter combinations (Table 2). Each simulated net pen failure introduced 4,000 YOY cobia to the native population, our simulations included f between 0 and 10 per year (0 – 40,000 escaped cobia per year). Current efforts in the Caribbean use an average of 14 fish as broodstock (Benetti et al. 2008; Benetti et al. 2010); our simulations considered $\underline{N}_{\underline{brood}}$ values between 2 and 50 total individuals. Previous population genetic analyses in our lab have documented both small-scale genetic differentiation among cobia stocks in the western Atlantic (Darden et al. 2014) and more substantial global population genetic structure (SCDNR, unpublished data). The eight possible S populations in our simulations were chosen to span the geographic range of cobia, including populations in the Gulf of Mexico, Atlantic Ocean, and Indo-Pacific Ocean.

All simulations for this project were deployed in a parallel implementation on the College of Charleston's high performance computing cluster so that large numbers of replicate simulations could be conducted for each parameter combination. During each simulation, the entire Gulf of Mexico system was initialized with allele frequencies at each of the ten simulated microsatellite loci equal to previously determined empirical values (Darden et al. 2014; SCDNR, unpublished data). Sample sizes used for allele frequency estimation ranged from 38 to 824, but all non-Indo-Pacific populations had more than 70 individuals sampled (Table 3; SCDNR, unpublished data). Our simulations included a burn-in phase, where the model was simulated for 50 years to allow the system to reach equilibrium. Following the burn-in period, net pen failures were simulated for an additional 50 years to determine the genetic impacts on the Gulf of Mexico cobia population. Simulations with low-mortality of released fish were repeated between 28 and 300 times per parameter combination (Table S1), with fewer replicates for the Gulf of Mexico source population (28 per parameter combination) and simulations with f = 0 (at least 83 per parameter combination for all non-Gulf source populations). High-mortality replicates focused on broodstock collected from the Indo-Pacific (Thailand) or the inshore South Carolina population to capture the range of impacts seen in the larger simulation study. Each parameter combination was simulated twelve times to assess the impact of high early-life mortality on our results (Table S2). In total, 14,254 simulations were conducted across parameter combinations. R scripts for model specification and simulation are available from the authors on request.

Data Analysis

We examined changes in five genetic response variables in the Gulf of Mexico population over the course of our simulations. We tracked the average summed frequency of non-native alleles across loci, the level of genetic diversity in the Gulf of Mexico (summarized using Nei's gene diversity, H_e; Nei 1975), and the proportion of the total allelic diversity composed of alleles introduced to the Gulf of Mexico via net pen failures. These three statistics were recorded yearly over the course of our simulations. We also measured the levels of genetic differentiation that accumulated over the course of 50 years of net pen failure in the Gulf of Mexico. To this end, two genetic divergence measures, the exact test of genic differentiation (G) and F_{ST}, were calculated for the Gulf of Mexico cobia population across two windows of time. For the first time window, differentiation statistics were calculated from comparisons of the population at the start of the simulations (year 0) and at the end of the 50-year burn-in phase (year 50). These same statistics were also calculated for comparisons between the population at the end of the burn-in phase (year 50) and the end of the simulated net pen failure phase (year 100). Levels of differentiation were then compared to determine if net pen failure (period 2, years 50 to 100) produced greater genetic differentiation than expected due to the effects of genetic drift acting over a similar length of time (period 1, years 0 to 50). In cases where the distribution (across replicates) of differences between F_{ST} values calculated for the two time periods overlaps zero, our simulations show no evidence of additional divergence driven by net pen failure. By contrast, if consistently greater $\underline{F_{ST}}$ values are seen for comparisons made over time period 2, then our data suggest a significant effect of net pen failure on the genetic composition of the native cobia stock.

Sensitivity Simulations

In order to determine if uncertainty in the demographic parameter values used in our model (e.g., juvenile and adult survivorship, age-specific fecundity) might bias our results, we conducted additional simulations to examine the sensitivity of model outputs to changes in several parameters. To this end, we chose a subset of the parameter space explored in the projection simulations, including the Indo-Pacific broodstock sources, but limited to only the largest broodstock number simulated (50 individuals). Perturbations of survival and reproductive rates were conducted for young and old age-class groupings, young adults were defined as all age classes younger than age 9 while mature adults were defined as individuals age 9 and older. Perturbations consisted of increasing survival rates or per capita fecundity by 1% for all age classes in each group. Survival and fecundity were perturbed separately in young and mature adults, resulting in four life history trait perturbation settings for our sensitivity simulations. Each combination was simulated 35 times, resulting in 140 additional simulations performed for sensitivity analyses.

Results

Effects on Genetic Diversity

Our simulations included two settings for early life survival of net pen escapees. Our assumption of negligible mortality is unlikely to hold in natural populations, but it represents an upper bound on the genetic impacts associated with a given f. Predictably, high early life mortality greatly reduced the impacts of escapement on the summed frequency of novel alleles and gene diversity (Supporting Figs. 1-4). In contrast, the proportion of the total alleles that are newly introduced by net pen escape showed less influence of the assumed early life mortality rate in our simulations (Supporting Figs. 5 and 6). In other words, many novel alleles were still introduced to the system, but the frequencies that they attained in our simulations were substantially reduced with high mortality early in life. Qualitatively, the patterns evident in our simulation outputs were similar between high and low early life mortality simulations. Conservatively from a management standpoint, we focus on simulations with low early life mortality and present results from these cases exclusively below.

As expected, higher f resulted in greater genetic impacts on the native cobia population. The range of f simulated in our project resulted in total escapee numbers ranging from 4,000 (1 net pen failure) to 40,000 (10 net pen failures) fish per year. Under higher f, the summed frequency of novel alleles at the end of our simulations increased from < 1% to > 3% for the largest simulated N_{brood} (Fig. 2; Supporting Fig. 2). Similarly, gene diversity increased with additional failures (Fig. 2; Supporting Fig. 4), but the proportion of alleles that were introduced showed less influence of the simulated f (Fig. 2; Supporting Fig. 6). As seen for high vs. low early life mortality, these results indicate that increased f had comparatively little impact on the total number of novel alleles introduced to the system. Rather, the number of novel alleles introduced to the Gulf of Mexico was more sensitive to N_{brood} and S, particularly as N_{brood} increased from its minimal simulated value.

All three of the genetic diversity statistics monitored in our simulations were impacted by the simulated $\underline{N_{brood}}$ value. The summed frequency of introduced alleles, overall gene diversity, and the proportion of introduced alleles all increased with larger $\underline{N_{brood}}$ (Fig. 2). Gene diversity and summed allele frequencies increased linearly over the course of our simulations, but the majority of the increase in the proportion of non-native alleles occurred early in the net pen failure period, and the steepness of the slope over the first few years of net pen failure was greatest in simulations with the highest $\underline{N_{brood}}$ (Supporting Fig. 6).

Our simulations considered eight potential $\underline{S}$ populations that differed substantially in their genetic divergence from the cobia population inhabiting the Gulf of Mexico (Table 3; SCDNR, unpublished data). Specifically, estimates of $\underline{R_{ST}}$ (which are influenced by both differences in allele frequencies and differences in the size of microsatellite alleles between populations) were at most 0.013 between cobia populations in the Gulf and Atlantic, but ranged from 0.494 to 0.611 for comparisons between Gulf of Mexico and Indo-Pacific populations (Table 3; SCDNR, unpublished data). Gulf of Mexico and western Atlantic broodstock sources resulted in only minor changes in the genetic metrics tracked in our simulations (Supporting Figs. 2, 4, and 6). In contrast, use of broodstock from Indo-Pacific populations produced sizeable changes in average summed novel allele frequencies (as high as 3% with the highest $\underline{f}$ and $\underline{N_{brood}}$ values; Fig. 2), gene diversity (increases of ~0.04; Fig. 2), and the proportion of non-native alleles in the Gulf of Mexico population (up to ~12%; Fig. 2). The influences on each of these response variables were directly related to the observed levels of differentiation between the Gulf of Mexico cobia population and the population chosen as a source of broodstock

(Supporting Fig. 7). Thus, levels of genetic differentiation between the source population and the native population were paramount in determining the magnitude of genetic impacts associated with net pen failure.

We also examined patterns in the standard deviation of genetic response variables across simulated replicates. Both the average frequency of introduced alleles (Supporting Fig. 8) and the average gene diversity in the Gulf of Mexico (Supporting Fig. 9) showed more variation among replicates with higher $\underline{f}$. Additionally, variation among replicates for these two metrics also showed decreasing trends as $\underline{N_{brood}}$ increases (though not as striking as the increases associated with higher $\underline{f}$). These patterns are expected, as higher $\underline{f}$ tends to increase the magnitude of the mean response (e.g., Supporting Figs. 2 and 4) and a more representative sample of the source population is achieved by increasing $\underline{N_{brood}}$, thus the among replicate variation is decreased by adding additional broodstock individuals. In contrast to the other metrics, the proportion of novel alleles displayed elevated variation only under low $\underline{N_{brood}}$ (Supporting Fig. 10), reflecting the primary influence of $\underline{N_{brood}}$ on the slope of the mean proportion of novel alleles. All simulations for a particular $\underline{S}$ population with $\underline{N_{brood}}$ of ten or more individuals reached an asymptote at approximately the same proportion of novel alleles (Supporting Fig. 6).

Effects on Temporal Genetic Differentiation

In order to determine if 50 years of net pen failure produced a discernible signal of temporal population differentiation in our simulations, we compared levels of genetic divergence between the beginning and end of the burn-in period (years 1-50) with those seen after 50 years of net pen failure (years 50-100). These comparisons were made using both the exact test for genic differentiation (G) and F-statistics (F_{ST}). In cases where the distribution of differences between statistics calculated across these two windows of time overlaps zero, net pen failures produced no more genetic differentiation than seen over the course of 50 years of genetic drift. Note that without net pen failures, the difference between values over the burn-in and simulation periods tends to be slightly less than 0, indicating more differentiation during the first 50 years of the simulation than over the remaining 50 years (Supporting Fig. 11). The divergence over the burn-in period is likely the result of a "filling in" of the allele frequency distribution as new alleles (that fill gaps in the empirical distribution of allele sizes) are created by mutation early in the simulations. However, with f = 10 failures per year and an N_{brood} = 50 individuals, a clear signal of changing genetic composition in the native population was evident, even when using a genetically similar S population (Supporting Fig. 12).

Low f and small N_{brood} resulted in similar levels of genetic differentiation to that seen over the course of 50 years without net pen failures (Fig. 2). However, as N_{brood} increased, the influence of S was evident even at low f (Supporting Figs. 13 and 14). With higher f (moving up the columns of Supporting Figs. 13 and 14), successively greater levels of genetic differentiation occurred. In these cases, the effects of N_{brood} and S were also apparent. Larger N_{brood} resulted in greater differentiation, particularly when using Indo-Pacific S populations for net pen aquaculture in the Gulf of Mexico. Although minor compared to Indo-Pacific sources, significant temporal genetic differentiation also resulted from using broodstock from Atlantic populations, particularly with high f and large N_{brood} (Supporting Figs. 13 and 14). Patterns of temporal differentiation under different f, N_{brood}, and S were consistent for the two statistics calculated (Supporting Figs. 13 and 14).

Sensitivity Analysis

Life history parameters employed in our model were based on recent data from stock assessments conducted for the Gulf of Mexico cobia population (SEDAR 2013). Nonetheless, we conducted sensitivity analyses to determine if our results were robust to minor uncertainties in demographic parameter values. For all genetic metrics tracked, modifications of vital rates did not result in substantial changes in the outputs of our simulations (Supporting Fig. 15). The results of our sensitivity analyses indicated that our model was highly robust to minor uncertainties in demographic rates. However, this sensitivity analysis may be influenced by the relatively static demographic trajectories under the simulated parameter modifications. Specifically, the demographic rates modeled produced a population growth rate (») greater than 1, in both forecasting and sensitivity analysis parameterizations. When population size in the simulations exceeded carrying capacity, individuals were randomly removed irrespective of their age. Thus with » > 1 in both cases, there was little influence of vital rate modifications on the overall demographic trajectory of the population, even though the age-structure of the simulated population was impacted. Thus, our sensitivity analyses effectively represent the influences of age structure modifications on the genetic metrics tracked in our simulations. It is likely that larger effects on genetic response variables would result if demographic rates in the model were set to yield » < 1. The results of our model, in terms of absolute changes in the genetic diversity metrics tracked in our simulations, are undoubtedly highly sensitive to our assumption of the size of the cobia population in the Gulf of Mexico. However, the qualitative patterns identified here (i.e., faster accumulation of novel alleles under simulations with larger $\underline{N}_{brood}$, greatest influence of $\underline{S}$) should be robust to changes in the total population size. Effectively, increasing the size of the wild population would be equivalent to reducing $\underline{f}$ or increasing mortality of escaped fish. For this reason, carrying capacity was not altered in our sensitivity analysis simulations.

Discussion

In recent years, great interest has developed in the expansion of offshore net pen aquaculture in the U.S., with particular emphasis on cobia as a candidate species for offshore grow out. Given the potential adverse consequences (both ecological and genetic) of escapement from offshore net pen facilities (Naylor et al. 2005; Thorstad et al. 2008; Jensen et al. 2010), it is imperative that proactive best management practices are developed prior to the widespread construction of these facilities. In this study, we integrated empirical data on cobia life history and genetic structure in an individual-based simulation model to assess the potential changes in wild population genetic composition that might result over a management-scale time frame from the failure of offshore cobia net pen aquaculture facilities in the Gulf of Mexico.

Conceptually, net pen failure is similar to captive breeding for population supplementation and stock enhancement. In both cases, offspring from a relatively small number of adults are overrepresented in the wild population, thereby increasing the variance in reproductive success and (typically) reducing effective population size. Both stock enhancement and captive population supplementation have been the subject of substantially more research than net pen failure (but see Volk et al. 2015). Previous authors have largely focused on the impacts of supplementation on effective population size (e.g., Ryman and Laikre 1991; Tringali and Leber 1999; Wang and Ryman 2001), inbreeding levels (Duchesne and Bernatchez 2002; Oota and Matsuishi 2005), or population fitness (Lynch and O'Hely 2001; Volk et al. 2015). Analytical and simulation studies have shown increasing genetic effects with smaller hatchery effective sizes (Ryman and Laikre 1991; Waples and Do 1994; Tringali and Leber 1999; Lynch and O'Hely 2001; Duchesne and Bernatchez 2002; Oota and Matsuishi 2005) and greater contribution rates of stocked fish (Ryman and Laikre 1991; Duchesne and Bernatchez 2002; Oota and Matsuishi 2005).

Our results agree with previous research showing greater genetic changes with higher stocking rates (similar to increases in $\underline{f}$ in this study), but also illustrate the importance of considering the source of broodstock used for hatchery production in net pen aquaculture systems. In fact, $\underline{S}$ had by far the greatest influence on the genetic diversity and differentiation metrics tracked in our simulations. Furthermore, data from our simulations highlight the more rapid influx of non-native alleles that is possible with larger broodstock population sizes in failure-prone net pen aquaculture operations. Thus, if native source populations are not available for net pen aquaculture, careful consideration should be made when deciding on targeted $\underline{N_{brood}}$ for hatchery production of farmed fish.

The primary goal of our research was to use population genetic simulations to provide guidance and recommendations to managers of net pen aquaculture operations. Our individual-based modeling framework allowed us to accurately simulate annual genetic responses in a long-lived species with overlapping generations while also incorporating demographic stochasticity.

The model can be tuned to match the life history and population genetics of any species of interest, and provides real-world quantitative predictions concerning the genetic outcomes associated with net pen failure. These aspects make our model better suited for adaptive management of net pen aquaculture than more computationally efficient alternatives (i.e., mathematical equations or coalescent simulations), due to its ability to accurately portray age structure in a population with overlapping generations across an ecological time scale that is meaningful for management.

Our simulation results illustrated the simultaneous influences of $\underline{f}$, $\underline{N_{brood}}$, and $\underline{S}$ on levels of genetic diversity, frequencies and numbers of introduced alleles, and temporal genetic differentiation within the native cobia population. Increases in $\underline{f}$ and $\underline{N_{brood}}$ both led to increases in the summed frequencies of introduced alleles, larger numbers of introduced alleles, and greater temporal changes in the genetic composition of the cobia population. As expected, more differentiated $\underline{S}$ populations produced stronger impacts on genetic diversity. The highest $\underline{f}$, largest $\underline{N_{brood}}$, and most divergent $\underline{S}$ led to a substantial influx of non-native alleles, with introduced variants comprising ~12% of total allelic richness in the Gulf of Mexico and the average (across loci) summed frequencies of introduced alleles approaching 3%. The levels of observed introgression in simulations with these settings markedly altered allele frequencies in the Gulf of Mexico, as indicated by temporal comparisons following 50 years of net pen failure.

While our simulations were tuned to match life history and genetic data for cobia in the Gulf of Mexico, patterns evident in our results would likely apply to any species targeted for net pen aquaculture. Thus, we can derive general recommendations for responsible broodstock selection and maintenance for net pen aquaculture systems from simulations of the cobia-specific model. Of the model attributes varied in our simulations, $\underline{S}$ had the greatest impact on the composition of the wild population. Influences of $\underline{S}$ were readily apparent on each of the statistics tracked over the course of our simulations. Intuitively, higher $\underline{f}$ resulted in greater introduced allele frequencies, while larger $\underline{N_{brood}}$ had more influence on the rate at which novel allelic variants accumulated in the Gulf of Mexico. With larger $\underline{N_{brood}}$, a more representative sample of the source population's allelic variation is contained in the annual broodstock collections, and the fraction of the total allelic variation introduced through net pen failure saturates more quickly. Thus, when non-native source populations are chosen for broodstock collection, the typical recommendation to maximize the effective population size in the hatchery (Blankenship and Leber 1995; Lorenzen et al. 2010) by using large numbers of broodstock leads to the rapid introduction of novel allelic variants and substantial changes in the genetic composition of the population.

Some level of escape is unavoidable in offshore net pen aquaculture systems, but our simulation results suggest that designing proactive, environmentally-responsible best management practices for broodstock selection and net pen stocking can allow aquaculturists to minimize potential negative effects of aquaculture escapees on wild populations. Given the strong influence of $\underline{S}$ on all measured response variables, the use of exclusively local broodstock sources in net pen aquaculture operations would limit introgression of non-native alleles following net pen failure. For cobia in the Gulf of Mexico, the use of locally-sourced broodstock individuals that are regularly rotated on a short time interval (2-3 years) with large $\underline{N_{brood}}$ (> 20 individuals) should limit the genetic impacts of escapees on the wild population. While this scenario was not explicitly simulated in our study (i.e., broodstock were rotated yearly in all simulations), short-term retention of broodstock is far more practical from the hatchery management perspective. More generally speaking, genetic monitoring of wild and hatchery populations is also beneficial, before (i.e., to gain baseline knowledge on population structure and genetic diversity in the species of interest), during, and after implementation of net pen aquaculture. The archival of spawning design data, genetic data for all broodstock individuals, and subsamples of offspring stocked into net pens is a proactive approach to aid in identification of escapees and allow for quantitative evaluations of genetic diversity and effective size in hatchery-produced fish. The intent would be for these recommended practices to be coupled with management strategies that minimize genetic risks associated with fish propagation, such as systems that reduce loss of fish from net pens and redundant technologies to prevent net pen failure (i.e., reducing $\underline{f}$ in our simulation model). The combination of responsible broodstock selection and maintenance, genetic monitoring, and implementation of redundant escape prevention technologies will help to minimize the probability of adverse genetic impacts of net pen escapees on wild fish populations.

We did not model loci under selection or the impacts of escaped fish on population fitness (but see Ford 2002; Baskett et al. 2013; Volk et al. 2015). Instead, our simulation results were explicitly focused on changes occurring at neutral loci following net pen failure. Thus, introduced alleles are assumed to be selectively equivalent to current genetic variants, and do not increase (or decrease) in frequency due to natural selection. If any of these alleles were to carry a selective advantage, the influences of net pen failure on the overall genetic composition, and potentially the fitness, of the native population would be substantially greater. Notably, both the census size and effective population size of the wild population will play large roles in determining the magnitude of genetic impacts from net pen escapement, as these parameters influence the proportional contribution of net pen escapees to the system and equilibrium levels of genetic diversity, respectively. Our simulations did not consider variation in census or effective population size (within a given $\underline{N}_{brood}$), and all simulations assumed a large wild population of cobia in the Gulf of Mexico. The modeling scenario and results presented here thus represent the most conservative (i.e., best-case) scenario of short-term genetic influences on native cobia populations in the event of net pen failures. Because our conservative assessment detected substantial genetic impacts under particular parameter combinations, with large influxes of non-native alleles and significant temporal genetic differentiation in the native Gulf of Mexico cobia population, implementation of best management practices for net pen aquaculture can minimize the genetic effects that aquaculture escapees have on wild populations. As both the ecological and aquaculture landscapes are constantly changing, we recommend that assessments of the genetic impacts of escapees from net pen aquaculture operations are repeated on a regular interval to continue to evaluate the potential influences escaped fish may have on native populations under these changing conditions. While the long-term impacts of net pen escapement are also important, our individual-based model is not appropriate for evolutionary time scale evaluations due to both its computational complexity as well as the loss of precision in simulation-based projections with increasing time. However, future studies that overcome current technological challenges while incorporating the reality of complex life histories and empirical data to address the long-term genetic influences of net pen escapement would be an excellent complement to our ecological-scale assessment.

While our model was developed specifically for cobia in the Gulf of Mexico, the recommendations provided by our research are general, and should apply to a variety of candidate species for net pen aquaculture. Nonetheless, it is advisable to undertake similar modeling exercises before applying specific results from cobia to other species as life history characteristics, overlapping generations, and population size all significantly influence the magnitude of genetic impacts. In adapting our recommendations, it is essential that levels of genetic structure between potential sources of broodstock and the wild population are considered. While the collection of life history data is difficult for many species, regional stock assessments compile these data to estimate demographic parameters for fisheries management purposes. Estimates from these assessments can then be combined with empirical data on the spatial distribution of genetic variation, as in our study, to forecast the impacts of aquaculture escapees. The individual-based simulations conducted here could easily be repeated prior to implementation of a proposed broodstock management plan to predict impacts on native genetic diversity under a variety of possible net pen failure rates. These simulations would provide valuable data on the expected outcomes following net pen failures and may help managers determine appropriate practices that simultaneously meet financial, operational, and conservation goals.

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Tables

TABLE 1. Life history information used to simulate Gulf of Mexico cobia population dynamics. Age-specific fecundities (b_x) are relative values based on average weights of females in different age classes. Age-specific survival probabilities (s_x) are derived from stock assessment data (SEDAR 2013).

Age (x)	s_x	b_x
1	0.059	0.000
2	0.407	0.046
3	0.515	0.151
4	0.562	0.314
5	0.600	0.429
6	0.615	0.543
7	0.615	0.629
8	0.637	0.714
9	0.636	0.800
10	0.639	0.857
11	0.642	0.886
12	0.636	0.914
13	0.636	0.943
14	0.636	0.971

15	0.636	0.971
16	0.636	1.000

TABLE 2. Parameter values used in model simulations. A fully factorial experimental design was adopted, wherein all possible combinations of broodstock source, broodstock number, and net pen failure rate were simulated to clarify their influences on the genetic impacts of net pen failure.

Parameter	Values Simulated
Number of net pen failures per year	0, 1, 5, 10
Number of broodstock fish (total)	2, 10, 20, 50
Broodstock Source Population	Gulf of Mexico, SC inshore, SC offshore, NC offshore, VA inshore, Thailand, Australia, India

TABLE 3. Source populations considered in our simulation study. For each source population, the sample size used for allele frequency estimation and the observed level of genetic divergence from the Gulf of Mexico cobia population (measured with R_{ST}; Slatkin 1995) are reported (SCDNR, unpublished data).

Broodstock Source	n	R_{ST}
Gulf of Mexico	285	--
South Carolina (Offshore)	411	0.008
South Carolina (Inshore)	824	0.014
North Carolina (Offshore)	194	0.009
Virginia (Inshore)	73	0.011
Australia	38	0.495
Thailand	109	0.590
India	48	0.594

Figures

FIGURE 1. Conceptual diagram of the cobia net pen failure simulation model. Each circle represents a population in the simulation. Arrows connecting circles correspond to movement of individuals (i.e., broodstock collection and net pen failure).

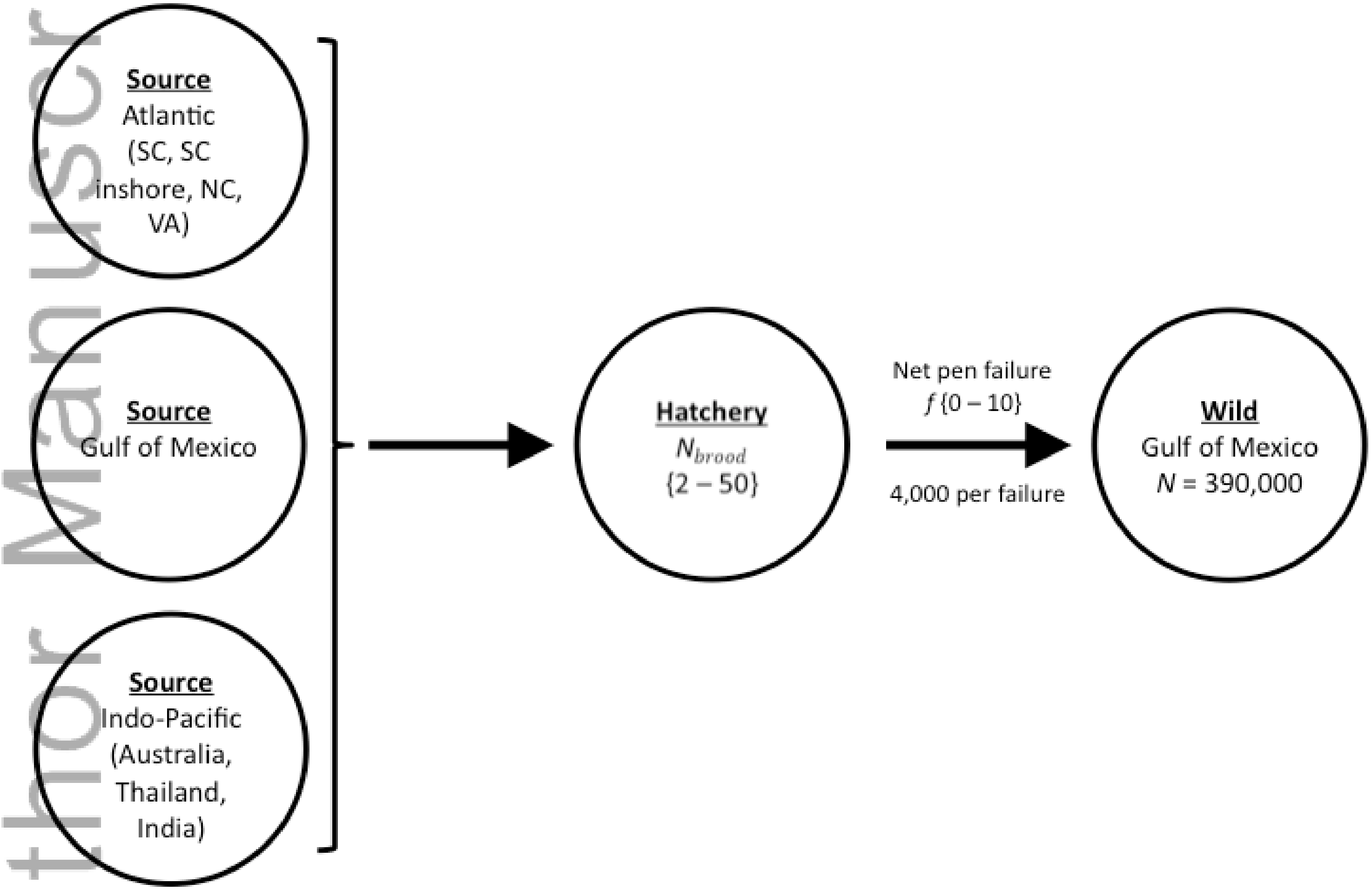
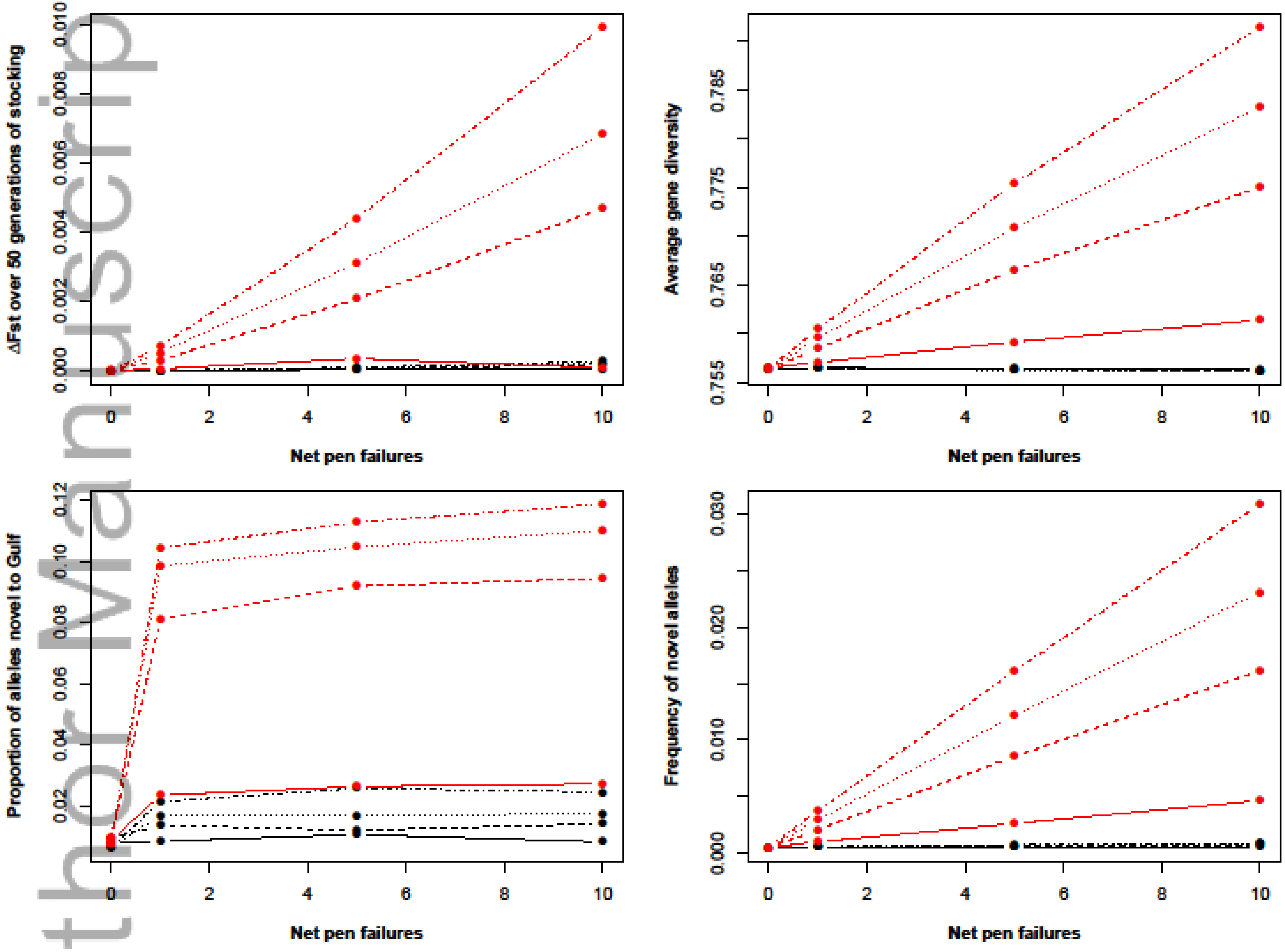

Source
Atlantic
(SC, SC inshore, NC, VA)
Source
Gulf of Mexico
Source
Indo-Pacific
(Australia, Thailand, India)
Hatchery
N_{brood}
{2 – 50}
Net pen failure
f {0 – 10}
4,000 per failure
Wild
Gulf of Mexico
N = 390,000

FIGURE 2. Genetic effects of net pen escapees on the wild Gulf of Mexico cobia population. Panels show (from left to right and top to bottom) the excess temporal genetic divergence due to 50 years of net pen failure, the average gene diversity ($\underline{H}_e$), the proportion of the total allelic richness comprised of non-native alleles, and the average summed frequency of non-native alleles following 50 years of net pen failure. Response variables are shown for a representative Atlantic source population (SCO; black lines) and an Indo-Pacific source population (AUS; red lines). Results are plotted separately for the four different numbers of broodstock simulated (2 - solid line; 10 - dashed line; 20 - dotted line; 50 - dotted-dashed line).


ΔFst over 50 generations of stocking
0.000
0.002
0.004
0.006
0.008
0.010
0
2
4
6
8
10
Net pen failures
Average gene diversity
0.755
0.765
0.775
0.785
0
2
4
6
8
10
Net pen failures
Proportion of alleles novel to Gulf
0.02
0.04
0.06
0.08
0.10
0.12
0
2
4
6
8
10
Net pen failures
Frequency of novel alleles
0.000
0.010
0.020
0.030
0
2
4
6
8
10
Net pen failures