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25. With a larger sample and fewer runs in the WLH-50 analysis, randomness can be rejected at $P = 0.05$. Ngandong is significantly closer to WLH-50, which is compatible with dual ancestry but is a refutation of the replacement hypothesis, so the consequences are the same even though the significance test results were opposite.
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Natal Homing in a Marine Fish Metapopulation

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Identifying natal origins of marine fishes is challenging because of difficulties in conducting mark-recapture studies in marine systems. We used natural geochemical signatures in otoliths (ear bones) to determine natal sources in weakfish (*Cynoscion regalis*), an estuarine-spawning marine fish, in eastern North America. Spawning site fidelity ranged from 60 to 81%, comparable to estimates of natal homing in birds and anadromous fishes. These data were in contrast to genetic analyses of population structure in weakfish. Our findings highlight the need for consideration of spatial processes in fisheries models and have implications for the design of marine reserves in coastal regions.

The exchange of individuals among geographically separated groups, or connectivity, is a critical property of marine populations (1). Connectivity rates determine colonization patterns of new habitats, the resiliency of populations to harvest, and the design of marine protected areas (MPAs). Quantifying exchange rates in marine organisms is, however, extremely difficult because natal origins of adults are almost invariably unknown (2, 3). This lack of knowledge is primarily due to the difficulty of conducting mark-recapture studies in species that are characterized by the production of large numbers of small pelagic offspring that suffer high initial mortality rates. Recently, tagging techniques using natural isotopic and elemental markers have been developed for species that were not

able to be tagged or recaptured using conventional approaches. Use of these natural tags may, therefore, allow questions of natal origins, spawning site fidelity (or philopatry), and return migrations to be addressed in organisms and environments for which we have little information, including migratory songbirds (4), butterflies (5), and fish (6, 7). For instance, fisheries ecologists have yet to determine rates of natal homing in any species of marine fish, even though spawning site fidelity and other complex migratory behaviors are common in birds (8) and anadromous fishes (9). We provide unique estimates of philopatry and population structure in weakfish (*Cynoscion regalis*) using stable isotope and elemental signatures in otoliths of returning spawners as a natural tag of natal origin.

Adult weakfish follow an annual migration pattern along the east coast of the United States that takes them from overwintering grounds south and offshore of Cape Hatteras to spawning locations in estuaries and coastal embayments throughout the species range (Florida to Maine) in the spring and early summer. Larvae are generally retained within natal estuaries through selective tidal stream transport (10), and they reside in these estuaries until migrating to overwintering

grounds in the autumn. Given the lack of larval dispersal, connectivity rates are primarily determined by the propensity for adult fish to return to their natal estuary to spawn. Previous studies of weakfish population genetics using allozymes and mitochondrial DNA (mtDNA) (11, 12) have found no evidence of genetic differentiation. However, genetic approaches may not have sufficient resolution to quantify natal homing unless straying is negligible over evolutionary time scales. The natural tag approach that we took provided estimates of natal homing and population structure in the presence of significant connectivity among groups within the larger metapopulation.

The otoliths of teleost fish are accretionary structures located within the inner ear, and are composed primarily of aragonite deposited on a proteinaceous matrix. The utility of otolith chemistry as a natural tag relies on three properties of otoliths (13). First, the deposition time of otolith material can be estimated by reference to concentric rings in otoliths that are routinely used in age estimation by fisheries biologists. Second, the metabolically inert nature of otoliths ensures that the aragonite mineralogy remains unaltered after deposition. Third, the calcium carbonate and trace elements that make up most (>90%) of the otolith are derived primarily from the ambient water, as modified by temperature. The isotopic and elemental composition of the otolith will, therefore, reflect the environmental characteristics of the water in which the fish lives. Because physical and chemical composition characteristics of water vary spatially, otolith geochemistry records the water mass characteristics specific to a particular natal area. The mechanisms generating variability in the signatures may not necessarily be restricted to environmental differences. Nonetheless, application of geochemical signatures in otoliths as a natural tag does not require the reconstruction of these environmental differences, only that the signatures are sufficiently robust to allow

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accurate classification of an unknown fish to its natal estuary. In an earlier study, we showed that geochemical signatures in otoliths of juvenile weakfish varied significantly among five estuaries along the east coast of the United States (14). Individual juveniles collected in 1996 were assigned to natal estuaries using linear discriminant function analysis (LDFA) parameterized with $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, Mg/Ca, Mn/Ca, Sr/Ca, and Ba/Ca values in otoliths with cross-validated accuracies of greater than 85%. Potential errors due to

interannual variations in these signatures among year-classes were minimized by collecting spawning fish in 1998 that were themselves spawned in 1996 (15).

Most weakfish reach sexual maturity at 1 year of age, and histological analyses of the 2-year-old females confirmed that these fish were in spawning condition (16). Residual analyses of geochemical signatures in otoliths showed distinctive patterns among locations for both juveniles and adults (Fig. 1). Genetic data from weakfish

implied a single, panmictic population from Florida to Maine (12). If this assessment were accurate, residuals from signatures in spawning fish would have been randomly distributed around zero. Instead, geochemical signatures from spawners were, in general, similar to signatures of juvenile fish collected from the same locations. Although these data suggested that natal homing was prevalent, perfect agreement would only be expected under conditions of complete philopatry. It was more likely that spawners from one estuary represented a mixture of fish sourced from several different locations. Therefore, we used LDFA, parameterized with the residuals from geochemical signatures in the juvenile fish collected in 1996, to classify 2-year-old spawners in 1998 to their natal estuary. We compared posterior probabilities of group membership derived from LDFA to assess the degree of concordance between geochemical signatures from juveniles collected within their natal estuary and adults classified to that estuary by LDFA (Fig. 2). Frequency distributions of posterior probabilities for both juvenile and adult weakfish were similar and positively skewed at all five locations; this finding suggests that the confidence with which adults were classified to a particular estuary on the basis of geochemical signatures was comparable to that of juveniles collected from their natal estuary.

The univariate and multivariate analyses confirmed that geochemical signatures in otolith cores from spawning 2-year-old weakfish matched well with the ground-truthed signatures from whole otoliths of juvenile weakfish collected in each of the five estuaries 2 years previously. Our ultimate goal was to determine the proportion of spawning fish at each of the estuaries that were themselves a product of that estuary, in order to quantify natal homing. The analysis did not require information on individual fish, but rather accurate estimates of the proportion of spawning fish in an estuary that were sourced from each of the five study estuaries. We used maximum likelihood estimation to derive these proportions, using an algorithm similar to that developed for mixed stock assignments from genetic data (17, 18). Homing of spawning weakfish to natal locations was high, ranging from 60% in Pamlico Sound to 81% in Georgia (Fig. 3). Straying was largely confined to locations adjacent to natal estuaries, and was not due to a complete breakdown of homing behavior. For instance, Delaware Bay strays were predominantly found in Chesapeake Bay, whereas strays from New York were only found in Delaware Bay. We know of no comparable data in any marine fish species with which to compare our estimates of natal homing. Mark-recapture studies on Atlantic herring (*Clupea harengus*)

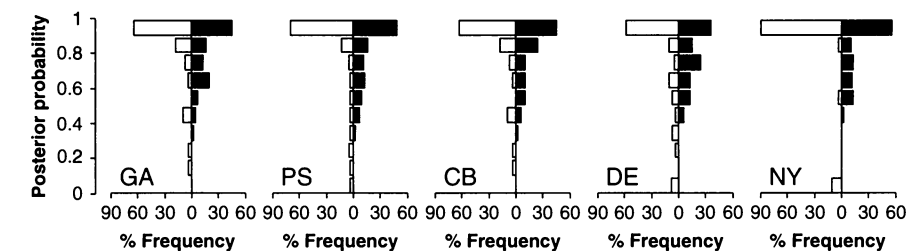
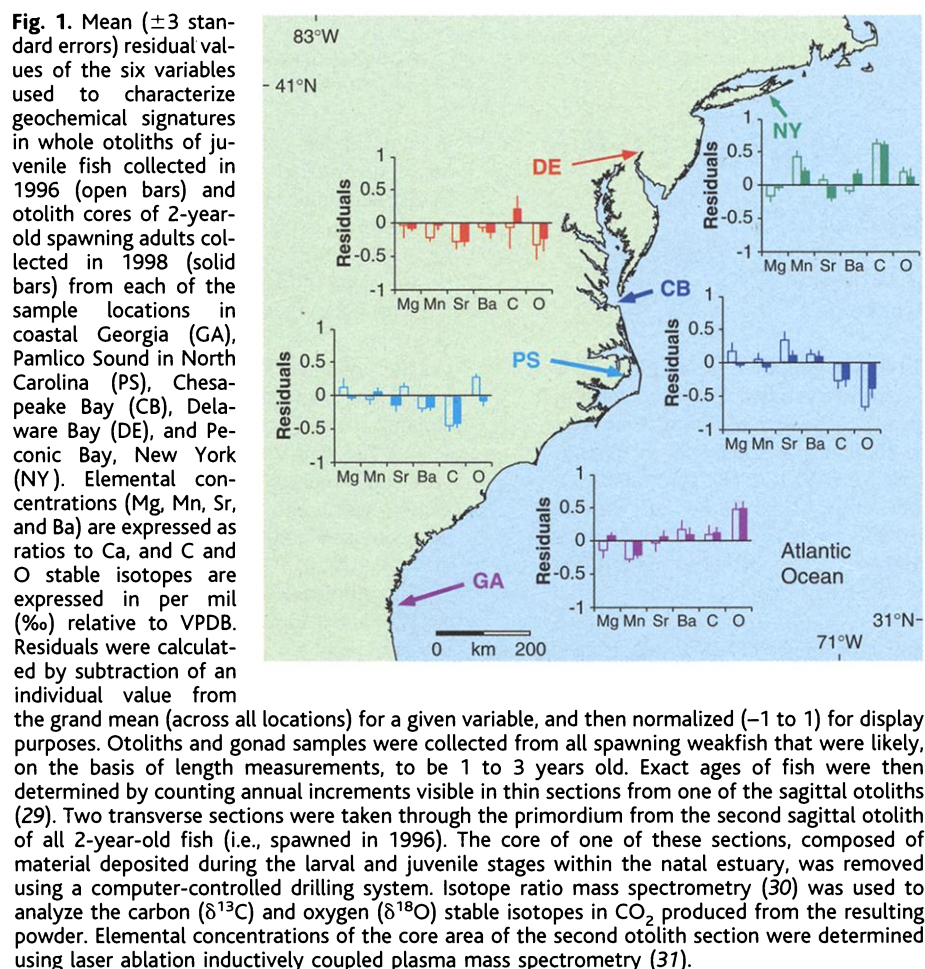


Fig. 2. Distributions of posterior group membership probabilities of juvenile weakfish collected within their natal estuary (open bars), and spawning weakfish classified to their natal estuary (solid bars), determined from LDFA of geochemical signatures in otoliths. These probabilities indicate the confidence with which individual juvenile or adult weakfish were classified to a particular estuary. Probabilities of less than 0.2 are possible only for the juveniles because classification accuracy to a natal estuary was less than 100%, and a minimum posterior probability of 0.2 was required before an adult was classified to a given estuary. Sample location codes are as in Fig. 1.

found that rates of repeat spawning, whereby adult fish tagged on the spawning grounds return to that same area to spawn in subsequent years, ranged from 75 to 95% (19). However, because the natal origin of these fish was unknown, it was impossible to determine the proportion of repeat spawners that were also homing to natal locations.

We can only speculate on the mechanisms by which individual fish may be able to navigate back to natal spawning sites some 2 years after initial outmigration from juvenile nursery areas. The ability of adult salmonids to home to natal streams through the use of olfactory and other cues is well known (20). Similar imprinting by juvenile weakfish while they reside in natal estuaries is possible, because juveniles may spend 3 to 5 months in these nursery areas before migrating out during the fall of their first year of life. Alternatively, these fish may learn of migration routes and spawning sites through social transmission or tradition, as has been suggested for Atlantic herring (19).

Weakfish are currently managed as a single stock along the east coast of the United States on the basis of allozyme and mtDNA data that have suggested no genetic structuring throughout the region. More recently, analyses of microsatellite and intron markers from the same juvenile weakfish used in the otolith assays detected no ge-

netic differentiation among the five estuaries (21). Our data showed that there is much more spatial structure than is currently assumed by fisheries managers, and that it may be useful to consider weakfish population dynamics from a metapopulation perspective. These results do not, however, contradict the genetic analyses. There is sufficient exchange, even among those estuaries with the highest levels of natal homing, to prevent genetic divergence. Nonetheless, the data highlight an inherent problem when fisheries management decisions are based on genetic approaches that are sensitive to extremely low rates of exchange (22).

The connectivity estimates that we derived can be used to parameterize metapopulation models of weakfish dynamics that may, in turn, be used to evaluate novel approaches to fisheries management. For example, the finding of significant spawning site fidelity has considerable implications for the design of MPAs along the east coast of the United States. Weakfish subpopulations with high levels of natal homing will be significantly more vulnerable to fishing activity than would be predicted on the basis of current stock models. Ensuring a scientific basis for the design and implementation of MPAs is critical if marine reserves are to be effective, because ultimately the credibility of agencies that promote and administer MPAs will be judged by the choice of suitable locations for protection (23). Tracing population connectivity through larval dispersal and natal homing will be the most important element in these efforts.

diately upon collection, and then were returned to the lab where otoliths and gonads were removed. Otoliths were cleaned of any obvious tissue and then stored dry in polypropylene vials. Gonads were fixed in 10% formalin for 24 hours, rinsed, and then transferred to 70% ethanol for storage before histological sectioning.

16. Histological analyses of female weakfish gonads were conducted following the procedure of (24). All 2-year-old fish were sexually mature. Moreover, 70% of the females were found either to be gravid and in the process of spawning (as evidenced by the presence of hydrated oocytes) or to have spawned very recently (as evidenced by the presence of postovulatory follicles).
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31. Data on element/Ca ratios from the cores of otoliths were collected on a Finnigan MAT Element2 inductively coupled plasma mass spectrometer equipped with a Merchantek EO LUV266X laser ablation system. The laser setup was similar to that described by (27), in which a wet aerosol is constantly aspirated into a spray chamber, where it is mixed with the carrier gas (He) from the laser cell before being transported to the plasma. Quantification followed the approach outlined in (28). Precision (relative standard deviation) of the technique, obtained by analysis of five replicate samples in a homogeneous aragonite solid, was as follows: Mg/Ca = 1.0%, Mn/Ca = 0.8%, Sr/Ca = 0.6%, and Ba/Ca = 0.8%.
32. Supported by the Recreational and Commercial Fishing Advisory Boards of the Virginia Marine Resources Commission, Virginia Sea Grant College Program grant NA56RG0489, and NSF grants OCE-9876565 and OCE-9871047 (S.R.T. and C.M.J.). We thank P. Geer, C. Grahm, T. Targett, L. Daniel, and J. Fortuna for weakfish samples; G. Mackenzie, A. Saied, and B. Wells for technical assistance; D. Naik for developing the maximum likelihood estimation procedure; and S. Campana, B. Gillanders, G. Jones, M. Kingsford, J. Hare, S. Swearer, and two anonymous reviewers for helpful comments on the manuscript.

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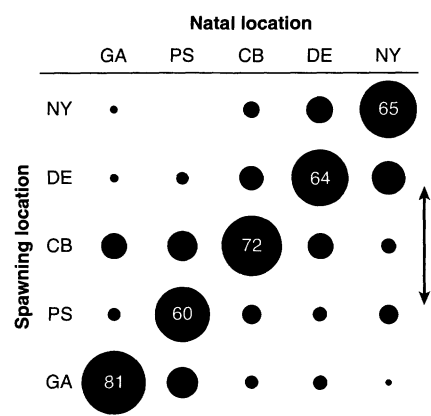


Fig. 3. Rates of natal homing in spawning weakfish (*Cynoscion regalis*). Bubbles are scaled by area to the percentage of fish from each of the five locations that were spawning in both their natal estuary (percentage shown in bubble) and each of the other four estuaries (arrow denotes direction in which proportions sum to 100% down the vertical axis). We initially determined the natal location of adult weakfish in each of five estuaries (location codes are as in Fig. 1) expressed as a percentage of the total number of spawners collected in that estuary, using maximum likelihood estimation. These data were then used to calculate the proportion of adults from a given natal location that had returned to that same location to spawn.

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