**Assessing stock structure of Pacific cod (*Gadus macrocephalus*) by combining ecological and evolutionary perspectives**

**Authors:**

Fisher, M.C.1

**Affiliations:**

1. School of Aquatic and Fishery Sciences, University of Washington, Seattle WA 98105, USA

**Abstract**

Population structure of harvested fish species is often assessed from a genetic perspective; however, knowledge of the actual distribution and migration of individuals which contribute to these evolutionary patterns is important for fishery managers, who work primarily on ecological timescales. We place genetic population structure in the context of individual life histories for Korean Pacific cod (*Gadus microcephalus*) stocks by analyzing otolith microchemistry data and comparing it with existing population genetic analysis. A total of 167 samples from six spawning groups around the Korean peninsula were analyzed for otolith microchemistry and SNP genetic markers. We first established that profiles composed of eight elements differed significantly between sampling sites, particularly Jinhae Bay, Pohang, and the Yellow Sea, through a PERMANOVA and by visualization in a non-metric Multidimensional Scaling (NMDS) ordination. This proves that otolith microchemistry can be useful in an entirely marine setting. A Mantel test showed that edge microchemistry was moderately positively correlated with core microchemistry, suggesting that most individuals were caught on spawning grounds with similar water chemistry as their natal spawning ground. However, visual comparison of individual clustering based on edge and core microchemistry, using both hierarchical agglomerative cluster analysis and kmeans clustering, showed less clustering between individuals caught at the same sampling site in the core microchemistry data. When compared to genetic data, otolith microchemical analysis was able to differentiate between spawning groups on a finer scale; while genetic analysis did not show differentiation within the southern coast or between temporal replicates, otolith data could be used to distinguish between temporal replicates both within and between years, as well as the Jinhae Bay site from all other southern sites.

**Introduction**

Fisheries management plans that include population genetic structure reduce the probability of overexploitation of local populations and allow for a more sustainable yield. Additionally, evolving threats to fisheries species from climate change have stressed the importance of preserving standing genetic variation and locally adapted populations, both of which can promote species resilience. As next-generation sequencing methods allow us to determine genetic structure on much finer scales in species characterized by high gene flow and large population size, the application of population genetic research to the management of purely marine species becomes more feasible.

Yet we still have much to learn about the ecological context in which population genetic patterns evolve. This is especially true of marine species, which encounter fewer barriers to dispersal and tend to consist of large, geographically expansive populations. By combining population genetic approaches with ecological studies of individual life history, we can gain additional insight into the drivers of evolutionary patterns on ecological timescales in marine populations.

This may also provide marine fisheries managers with a more complete toolkit to identify and manage population structure. For example, genetic analyses only detect migration on evolutionary scales, and only when migrants are reproductively successful. Recent studies have suggested that migration without reproduction is more common in fish species than previously believed1. Skipped spawning, in which a mature fish with an annual reproductive cycle does not spawn during a given year, is now thought to characterize many species and populations of fish. While this may not have an effect on population structure on evolutionary timescales, it has a significant impact on the yearly composition of local populations and therefore resource management. Fisheries are primarily managed on ecological timescales, and so the connectivity of populations through individual movement and behavior is a key consideration when discussing spatial stock structure.

Clearly, there is a need to couple studies of individual life history with population genetics in marine fish species. In the marine environment, traditional mark-recapture studies used to characterize individual behavior are limited in size and scope. Otolith microchemistry provides an alternative to tagging. Otoliths accumulate layers of calcium carbonate throughout a fish’s life, beginning in early larval stages. The layers incorporate some elemental chemistry from the body of water surrounding the fish. Otolith microchemistry can therefore be used as a type of “flight path” when matched to chemical profiles of water bodies. Previous studies have successfully combined genetic and otolith microchemistry data sets to distinguish between populations of Patagonian toothfish caught off of South America and Antarctica2, to prove limited mixing between black rockfish populations3, and to provide complementary information on the natal origin and genetic structure of a migratory cyprinid species4.

However, such studies used 20 or fewer microsatellites or allozymes for genetic analyses. Next-generation sequencing methods, which generate thousands of loci for finer-scale genetic population structure analysis, provide a more complete picture of population genetic structure which may be more conducive to ecological comparisons. Additionally, otolith microchemistry is often applied to estuarine or anadromous species, or across large geographic distances, to ensure spatial differences in elemental profiles. Otolith microchemistry applications to marine species is comparatively limited, primarily because of the expected homogeneity in element concentrations in the marine environment5.

Pacific cod is an excellent case study to address such questions. A marine finfish species which supports major fisheries across its North Pacific range, Pacific cod form coastal spawning aggregates during the winter months to breed. While such aggregates can be in close geographic proximity, previous genetic analyses suggest that Pacific cod display distinct population structure across its range6–9. This population structure has been proven to be critical to sustainable fisheries management of the species; a simulation study of Pacific cod stocks in Alaska found that establishing management units based on genetic research maintained stock sizes at target levels and even increased catch, while not managing for genetically distinct populations could reduce stock sizes below target levels (Spies & Punt 2015).

The maintenance of genetically distinct spawning aggregates is likely a result of some site fidelity and short larval dispersal distances, estimated from studies conducted along the western US and Canadian coastlines7. However, direct evidence of site fidelity in the form of individual migration is primarily derived from a single mark-recapture study conducted in the northwestern United States10. Field observations of non-spawning mature adults in Pacific cod spawning aggregates also raise the possibility that Pacific cod may display skipped spawning, a common behavior observed in their Atlantic relatives1. More direct evidence of site fidelity, and the linkage of individual behavior to genetic population structure, would provide needed insight into the biology of this species and greatly benefit Pacific cod fisheries management.

The purpose of this study was to explore Pacific cod population structure and site fidelity around the Korean peninsula according to otolith microchemistry, and to then compare these results from otolith microchemistry with next-generation sequencing data. In doing so, we address three key questions:

1. How different are otolith microchemical concentrations across Pacific cod spawning grounds around the Korean peninsula?
2. Is there evidence suggesting that Pacific cod return to their natal spawning ground during the spawning season in which they were caught?
3. What additional insights and management tools can be gained by comparing and/or integration next-generation genetic data with otolith microchemistry data?

**Methods**

*Sample Collection*

Tissue samples and otoliths were collected from 322 individuals across ten Pacific cod spawning aggregates in coastal waters around the Korean peninsula (**Table 1**). Three aggregates were sampled during the 2007 – 2008 winter spawning season, three aggregates during the 2014-2015 season, and one during the 2015 – 2016 season. Two additional spawning groups were sampled as temporal replicates, one at Geoje as a between-year replicate during the 2013-2014 season, and one at Jinhae Bay as a within-year replicate during the 2007-2008 season. Tissue samples were preserved in 95-100% non-denatured ethanol, stored at 4°C.

*Otolith Data Collection*

A total of 261 otoliths were scanned for thirteen trace elements using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) at the University of Oregon (**Fig. 2**). Element concentrations were then standardized against a calcium standard. Of the thirteen original elements, eight had relative concentrations that were large enough to produce a signal that could be distinguished from the calcium standard, and retained as “informative” for further analysis: boron (B11), barium (Ba138), Lithium (Li7), magnesium (Mg24), manganese (Mn55), lead (Pb208), strontium (Sr88) and zinc (Zn66). Otoliths were then aged and each year of growth partitioned into 4 additional sections (**Fig. 2**). Since multiple LA-ICP-MS measurements were taken within each section, concentration ratios were averaged together to reach a single concentration ratio for that section. This study utilizes the mean concentration ratios from measurements at the core of the otolith (Year 0; Section A), and from measurements at the edge of the otolith (Year varies by individual; Section D). Otolith core concentrations were assumed to be indicative of the natal spawning ground, and otolith edge concentrations of the spawning ground on which the individual was captured for sampling.

*Genetic Data Collection*

DNA was extracted from 322 fin clip and tissue samples using DNeasy Blood & Tissue Kits (Qiagen, Inc.). DNA was then quantified using Quant-iT PicoGreen dsDNA Reagent (Invitrogen, Carlsbad, CA) and visualized on an agarose gel. A total of 283 samples were prepared for RAD sequencing according to Baird et al. (2008) and Etter et al. (2011), with modifications to incorporate Agencourt AMPure XP beads (Beckman Coulter, Inc., Pasadena, CA) for purification (Gruenthal et al. in prep). Sequencing of 150bp single end (n = 157) and paired end (n = 126) reads was completed on an Illumina HiSeq 4000 (Illumina, Inc.) at the University of Oregon’s Genomics and Cell Characterization Core Facility.

Quality filtering and demultiplexing of raw RAD sequencing data, as well as de novo construction of a reference database of RAD loci, SNP discovery, and genotyping was completed using a combination of the Stacks 1.44 pipeline (Catchen et al. 2011; Catchen et al. 2013), Bowtie (Langmead et al. 2009), and NCBI’s Basic Local Alignment Search Tool, BLAST (Altschul et al. 1990), according to the procedures outlined in Gruenthal et al. (in prep) and Brieuc et al. (2013). A genotype file containing putative polymorphic SNPs present in ≥ 80% of fish per spawning aggregate was randomly filtered to include one SNP per RAD tag. Final filtering was then used to remove loci with minor allele frequencies (MAFs) with < 0.05 in all spawning aggregates, loci that did not conform to Hardy – Weinberg equilibrium, and loci missing more than 20% of genotypes in any of the spawning aggregates. Individuals were removed from the data set if they were missing genotypes at more than 30% of loci, or were found to be potentially contaminated using a combination of heterozygosity distribution and ML-Relate (Kalinowski et al. 2006).

*Data Subsetting*

After filtering out individuals which were not present in both genetic and otolith microchemical datasets, data were subset to only include individuals two years and older, according to otolith aging methods. Fish less than two years old are considered juveniles, and therefore not spawning fish, which are the subject of this study. Additionally, it is not known whether there is an ontogenetic shift in spawning migration patterns between adult and juvenile fish; therefore, the inclusion of juvenile fish in these analyses could lead to the mischaracterization of mature Pacific cod spawning behavior.

The size of the data set was further reduced for computational simplicity and to achieve more even sample sizes. Individuals were randomly removed from both Jinhae Bay 2007-2008 sample sets, so that the number of samples in each group reached n = 30. This left a total of 167 samples collected across five spawning aggregates, with temporal replicates taken at two sites (**Fig. 1, Table 1**).

*Statistical analysis of otolith microchemistry data*

Microchemistry data normality was assessed using Mardia’s multivariate normality test and QQPlot visualization with the R package MVN (cite). The data did not conform to multivariate normality. Data transformations, including natural log, log10, cube root, and square root transformations, did not normalize the data set. Thus the multivariate analytical methods (below) for this data do not include distributional assumptions.

Visualization of otolith microchemistry data by element and sampling site showed markedly different scales (**Fig. 3**), so each element’s concentrations were relativized by maxima using the R package vegan13. A Euclidean distance matrix was then calculated separately for the elemental ratios measured at the core of the otolith, and the elemental ratios measured at the edge of the otolith.

To determine whether elemental fingerprints differed significantly between the sampled Pacific cod spawning grounds, I performed a permutational analysis of variance (PERMANOVA) of the elemental ratios at the edge of the otolith against spawning site, with 100,000 permutations. To explore potential temporal differences in otolith microchemistry, I also performed a PERMANOVA of element ratios at the edge of the otolith for Jinhae Bay samples against spawning month, and Geoje samples against spawning year. I then conducted one-way ANOVAs for those elements which could be normalized to determine element-specific differences between sampling site, spawning year, and spawning month. This consisted of four elements: Barium138 (natural log-transformed), Magnesium24 (natural log-transformed), Strontium88, Zinc66 (natural log-transformed). I then ran a non-metric Multi-dimensional Scaling Analysis (NMDS) on elemental ratios from the edge of the otolith, and fit element vectors to the ordination using vegan13, to identify which elements were correlated with the separation of specific sampling sites across the ordination space. Although this step is commonly completed for otolith microchemistry analysis with a Linear Discriminant Analysis14–16, NMDS has been used when data do not conform to multivariate normality2. The NMDS was run with the wrapper function metaMDS in vegan13, with a maximum of 400 iterations and minimum of 40 runs (McCune & Grace 2002). In order to determine the optimum number of dimensions, “k”, I ran the NMDS with 1 through 7 dimensions (the number of sampling units), then evaluated a plot of dimensions against associated stress values. The minimum stress value was achieved with seven dimensions. However, a two-dimensional NMDS cannot accurately represent variation across seven dimensions. I therefore used three dimensions for the final NMDS, as this was the lowest number of dimensions from which I had no real risk of drawing false inferences (stress < 0.10; Clark 1998).

To approach the question of site fidelity, a Mantel test was used to determine whether there was a correlation between the elemental fingerprint at the otolith core and the otolith edge for each fish, which would indicate similar elemental signatures of the individual’s natal and final spawning ground. I ran the Mantel test using Pearson’s correlation coefficient and 100,000 permutations.

While the Mantel test may suggest correlation between the elemental fingerprint at the core and edge of the otolith, it is not possible to explore specific differences in groupings of individuals, or individual assignment to groups. Hierarchical polythetic agglomerative cluster analysis was run using the function hclust to explore groups of individuals based on otolith microchemistry at the core and the edge of the otolith. I first conducted the analysis with three separate clustering methods: the Ward method (ward.d2; Ward 1963), complete linkage (default), and the average or UPGMA method. Clustering produced using the Ward method most closely matched expectations and groups visualized in the NMDS ordination, and so was used for final analyses. With NbClust17, a gap index was calculated based on the Ward clustering method for both edge and core data sets. The best number of clusters and the optimal partition of individuals was determined based on the gap index, and a dendrogram generated for both core and edge microchemistry using the R package dendextend (Galili 2015). To compare clustering of individuals between the otolith edge and core datasets, I used a tanglegram, a common visualization in phylogenetics in which two dendrograms are drawn opposite each other, and then leaves connected with auxiliary lines (Scornavacca et al. 2011). The tanglegram function was called from the R package dendextend (Galili 2015).

*Analysis of genetic population structure and individual assignment*

The number of genetically distinct populations in the data was determined using STRUCTURE18. STRUCTURE places samples into groups whose members share similar patterns of variation using a Bayesian clustering algorithm with applied MCMC estimation. This allows for a progressive convergence toward reliable allele frequency estimates in each population, and membership probabilities of sampled individuals18. STRUCTURE was run using default parameters (burnin =50,000 and MCMC reps = 10,000) with K = 1 through 9 assumed populations, and 3 iterations per value of K. The likelihood of the data was calculated for each value of K, and then the mean log-likelihoods plotted to determine the optimum value of K (the smallest “stable” K value that maximizes the global likelihood of the data). The population assignment for each individual under the optimal value of K was then exported for comparison to otolith microchemistry.

*Comparison of genetic assignment and otolith microchemistry*

The first step in this comparison was to qualitatively assess the differences in spatial and temporal stock structure that are distinguishable from genetic analyses versus otolith microchemistry. I then visually assessed the groupings of individuals according to genetic assignment and otolith core microchemistry. To do so, I conducted an NMDS with the wrapper function metaMDS in vegan13, using the same parameters as with the edge microchemistry NMDS - a maximum of 400 iterations and minimum of 40 runs (McCune & Grace 2002). To determine the optimum number of dimensions, “k”, I ran the NMDS with 1 through 7 dimensions (the number of sampling units), then evaluated a plot of dimensions against associated stress values. The minimum stress value was achieved with seven dimensions, as in the NMDS conducted on edge microchemistry data. I again used three dimensions for the final NMDS, as this was the lowest number of dimensions from which I had no real risk of drawing false inferences (stress < 0.10; Clark 1998). I then coded point shape according to whether the individual’s genetic material was primarily assigned to the West or the South/Southeast population.

**Results**

*Elemental Fingerprints of Spawning Grounds*

A PERMANOVA of elemental fingerprints at the edge of the otolith by sampling site, spawning year (temporal sample at Geoje), and spawning month (temporal sample at Jinhae Bay) all showed significant differences in elemental fingerprints (*p* < 0.01; **Tables 2a-c**). However, the proportion of variance in elemental fingerprints that could be accounted for by each of these factors, rather than by residuals, differed. Whereas sampling site could explain 45% of the variance in elemental fingerprints, spawning year could only explain 13% and spawning month 14% of total variation (**Fig.**).

I was also able to conduct a one-way analysis of variance, or ANOVA, to explore element-specific differences between sampling sites, spawning year, and spawning month for four of the eight elements that could be normalized (**Tables 3-5**). All four elements – barium, magnesium, strontium, and zinc showed significant variation among sampling sites. Variation in magnesium concentrations across spawning years at Geoje was also significant, whereas there was a significant variation in barium concentrations across spawning months at Jinhae Bay.

A non-metric Multi-dimensional Scaling (NMDS) ordination provided insight into how element concentrations differed between sampling units. The NMDS, run with three dimensions, produced a stress value of 0.0934, which is associated with good representation of the data set (Clarke 1993), and a linear fit of R2=0.963 (**Table 8; Fig. 5**). The ordination showed distinct separation between the Jinhae Bay, Pohang, and the Yellow Sea samples (**Fig. 5**). Vectors indicating the fit of elements along the ordination axes suggest that boron (B11), barium (Ba138), and strontium (Sr88) concentrations lead to the differentiation of samples from Jinhae Bay. Samples from Pohang (2014-2015 spawning season) also appear to differentiate across the ordination space, correlated with magnesium (Mg24) and zinc (Zn66) concentrations, and somewhat correlated with lithium (Li7) and lead (Pb208). Although Geoje temporal samples (2014 and 2015), which were found to have significantly different elemental fingerprints in the PERMANOVA, did not differentiate from each other across the ordination space, this could be a result of other samples being much more highly differentiated (i.e. Pohang, Yellow Sea Block, and Jinhae Bay), or of variation of only two dimensions being represented in the NMDS visualization.

*Site Fidelity*

A Mantel test between distance matrices calculated from otolith core concentrations and otolith edge concentrations produced a Mantel R statistic of 0.3441, and a *p* value of 1 x 10-5. This suggests slight positive correlation between full elemental fingerprints of each individual’s natal spawning ground and the spawning ground at which they were caught.

Site fidelity was also visually explored through a cluster analysis, to determine if individuals that were caught on the same spawning ground would cluster together in an analysis based on core microchemistry. Hierarchical cluster analysis analyzed with the Tibshirani et al. (2001) gap statistic suggested that the data set was represented best by two clusters in both the otolith edge and otolith core datasets. In the otolith edge data set, one cluster was composed of approximately half of the Jinhae Bay samples, with the remaining samples across all sites in the second cluster; in the core data set, one cluster consisted of all Jinhae Bay samples as well as some samples from the Geoje and Yellow Sea sites, while the second cluster consisted of a mixture of samples from all sites except Jinhae Bay (**Fig. 6a&b**).

The tanglegram comparison of clustering between edge and core otolith microchemistry showed that some sampling sites had more variation among individuals in core microchemistry than others; however, with the exception of the Jinhae Bay site, individuals from different sampling sites were generally more intermixed when clustered according to core microchemistry (**Fig. 7a-d**). This was also evident when groupings suggested by cluster analysis were overlaid on the NMDS produced with edge microchemistry; hulls had far more overlap across sampling sites for core microchemistry than edge microchemistry (**Fig. 8a,b**).

*Genetic population structure*

STRUCTURE analyses suggested two genetically distinct populations, delineated between the western and south/southeastern coast. Assignment tests through the program primarily assigned individuals to the coast on which they were sampled. However, there were six individuals sampled from sites on the south/southeastern coast which had the majority of their genetic material assigned to the west coast: two fish from Pohang, two from Geoje (one from the 2013-2014 season, one from the 2014-2015 season) and three from Namhae.

*Genetic and otolith microchemistry comparisons*

The individuals from the south/southeastern population that were genetically assigned to the western population did not show otolith core microchemistry that could confidently conclude their true natal spawning aggregate was that of the Yellow Sea (YSBlock). When genetic population structure is overlaid on an NMDS of otolith core microchemistry (**Fig. 9**), there is not enough separation across the ordination space to identify outlier individuals in specific populations, except the Jinhae Bay spawning aggregates. However, one individual from the Pohang spawning aggregate which was genetically assigned to the west coast population is an apparent outlier on the NMDS. While this may be a result of individual variation in the uptake of water chemistry into the otolith, it is also possible that this individual derives from an unsampled spawning aggregate. The genetic assignment procedure will assign every individual to a given baseline, even if that baseline does not include the true population of origin.

In contrast to genetic data, which showed potential migrants from the west population to the southern coast, cluster analysis of otolith microchemistry showed several Yellow Sea samples clustering with the southern coast. However, fine-scale patterns in the cluster analysis should be interpreted carefully, given the low overall distinction between non-Jinhae Bay sites.

**Discussion***Otolith Microchemistry*

Otolith microchemistry has been primarily used to discern fish migratory patterns in estuary systems or in anadromous species. In this study, we prove that microchemistry can be useful in discerning between marine bodies of water, particularly along the coast. Not only did elemental fingerprint vary by sampling site location, but there was also significant evidence of temporal variation in element concentrations in PERMANOVAs. It should be noted that the spawning site explained a far greater proportion of total variation than either spawning year or month. However, follow-up ANOVAs suggest that this may be a result of the number of elements which varied significantly in their concentrations. Four out of the four elements tested showed significant variation among sampling sites. However, only one out of the four elements tested showed significant variation between spawning years and months.

When differences in sampling site elemental fingerprints were visually assessed through an NMDS, four sites became particularly distinguished across the ordination space. Samples from Pohang loaded higher than other sites on zinc and lead, two heavy metals. This is likely due to runoff from the port city of Pohang, which is a highly industrialized region. Jinhae Bay samples loaded high on strontium, barium and boron, all of which have been associated with salinity19. Jinhae Bay was a nearshore spawning aggregate along the southern coast of Korea, which consists of many fjord-like bays and inlets that may create a more heterogeneous salinity profile. However, the difference in element concentrations associated with salinity may also be a function of time period; the Jinhae Bay samples were taken during the 2007-2008 spawning season, whereas the Geoje, Namhae and Pohang samples were taken during either the 2013-2014 or 2014-2015 spawning season. Finally, Yellow Sea samples had higher Manganese concentrations. This could be a result of this site being the furthest offshore, or because this was the only site along the western, rather than the southern, coast. Additional samples from the western coast of the Korean peninsula could provide further insight into this pattern.

We were also able to see the significant difference in temporal replicates in the NMDS, especially between spawning months at Jinhae Bay. This is …

The significant variation in elemental fingerprints between spawning years and months in the temporal replicates indicates that element concentrations are measurably variable over time. Since individuals caught at the same sampling site encompassed a variety of cohorts (i.e. were of a variety of ages), this could explain why core microchemistry did not produce distinct clusters by sampling site or even region, despite genetic assignment tests suggesting that Pacific cod were most closely related to individuals on the spawning ground on which they were caught. Further analyses should look further into temporal variation.

*Otolith microchemistry v. Genetic assignment testing*

Genetic and otolith microchemistry analysis allowed for differentiation of spawning aggregates on different scales. Genetic data identified two highly differentiated populations. Of the two prior studies of population structure in Korean Pacific cod that were conducted with microsatellite loci (Gwak & Nakayama 2011; Kim et al. 2010), this study agrees with the most recently published manuscript which identified significant divergence between south and west coast spawning aggregates.

This was the first study to assess genetic differentiation between temporal replicates; Structure analyses suggested no significant divergence between spawning years or months at two of the sampling sites. Otolith microchemistry also suggested differences in elemental fingerprints between the western spawning aggregate, Yellow Sea, with the remaining samples. However, microchemistry was also able to distinguish between spawning aggregates within the south/southeastern population, particularly the Jinhae Bay and Pohang spawning aggregates. Otolith microchemistry also showed significant variation between temporal replicates, both between spawning years at Geoje and between spawning months at Jinhae Bay.

One of the most interesting conclusions that could be drawn from genetic and otolith comparisons is regarding the Jinhae Bay spawning aggregates. A temporal sample of different months (December and February) within the same spawning seasons was taken at this location because field observations suggested that two separate aggregates formed at different times during the spawning season. Genetic data show that on evolutionary timescales, these spawning groups are relatively homogenous. However, in cluster analysis of otolith microchemistry, these two spawning groups have quite distinct elemental fingerprints – and this pattern is replicated across both edge and core microchemistry (**Fig. 7c**). This suggests that ecologically, Pacific cod which spawn in Jinhae Bay are composed of two distinct groups: early and late spawning fish. However, there appear to be enough migrants between these groups that any distinction is drowned out on evolutionary timescales.

*Conclusions*

The ability to determine the natal spawning ground of an individual fish makes possible a wide range of studies on fish movement and behavior. It is encouraging to see a significant variation in elemental fingerprints between Pacific cod spawning grounds in this study. This is particularly true of Jinhae Bay and in the Yellow Sea, as previous work on otolith microchemistry has shown that strontium, barium and manganese are the most reliable geographic markers in marine species5. Thus there is strong potential for the use of otolith microchemistry to identify the natal spawning site for Pacific cod around the Korean peninsula.

Overall, the results support the literature suggesting that multidisciplinary approaches in marine species management can help managers ensure sustainability for species with complex demographic interactions and dispersal.20 dot dot dot

*Future directions*

While this study adds to the literature and contributes to fisheries management, it was limited by a lack of multivariate normality in the data set. Future research efforts should include a comparison of assignment tests based on otolith versus genetic data, using discriminant function analysis with jackknifing to assign individuals based on otolith microchemistry. This would allow for more conclusive verification of potential migrants identified through genetic analysis.

This study also did not exhaustively sample all spawning aggregates in Korean coastal waters. To confidently identify a single spawning ground, rather than a regional body of water, as a natal spawning site, a larger sampling effort would be required. Future efforts should also be made to characterize temporal variation in water chemistry at spawning aggregates.

**Sources**

1. Rideout, R. M. & Tomkiewicz, J. Skipped Spawning in Fishes: More Common than You Might Think. *Mar. Coast. Fish.* **3,** 176–189 (2011).

2. Ashford, J. R., Arkhipkin, A. I. & Jones, C. M. Can the chemistry of otolith nuclei determine population structure of Patagonian toothfish Dissostichus eleginoides? *J. Fish Biol.* **69,** 708–721 (2006).

3. Miller, J. A., Banks, M. A., Gomez-Uchida, D. & Shanks, A. L. A comparison of population structure in black rockfish ( *Sebastes melanops* ) as determined with otolith microchemistry and microsatellite DNA. *Can. J. Fish. Aquat. Sci.* **62,** 2189–2198 (2005).

4. Collins, S. M. *et al.* Population Structure of a Neotropical Migratory Fish: Contrasting Perspectives from Genetics and Otolith Microchemistry. *Trans. Am. Fish. Soc.* **142,** 1192–1201 (2013).

5. Sturrock, A. M., Trueman, C. N., Darnaude, A. M. & Hunter, E. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? *J. Fish Biol.* **81,** 766–795 (2012).

6. Canino, M. F., Spies, I. B., Cunningham, K. M., Hauser, L. & Grant, W. S. Multiple ice-age refugia in Pacific cod, Gadus macrocephalus: MULTIPLE ICE-AGE REFUGIA IN PACIFIC COD. *Mol. Ecol.* **19,** 4339–4351 (2010).

7. Cunningham, K. M., Canino, M. F., Spies, I. B. & Hauser, L. Genetic isolation by distance and localized fjord population structure in Pacific cod (Gadus macrocephalus): limited effective dispersal in the northeastern Pacific Ocean. *Can. J. Fish. Aquat. Sci.* **66,** 153–166 (2009).

8. Gwak, W. S. & Nakayama, K. Genetic variation and population structure of the Pacific cod Gadus macrocephalus in Korean waters revealed by mtDNA and msDNA markers. *Fish. Sci.* **77,** 945–952 (2011).

9. Kim, M.-J., An, H. S. & Choi, K.-H. Genetic characteristics of Pacific cod populations in Korea based on microsatellite markers. *Fish. Sci.* **76,** 595–603 (2010).

10. Rand, K. M., Munro, P., Neidetcher, S. K. & Nichol, D. G. Observations of Seasonal Movement from a Single Tag Release Group of Pacific Cod in the Eastern Bering Sea. *Mar. Coast. Fish.* **6,** 287–296 (2014).

11. Helser, T. (2016).

12. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10,** R25 (2009).

13. Oksanen, J. *et al.* *vegan: Community Ecology Packages.* (2018).

14. Barnett-Johnson, R., Teel, D. J. & Casillas, E. Genetic and otolith isotopic markers identify salmon populations in the Columbia River at broad and fine geographic scales. *Environ. Biol. Fishes* **89,** 533–546 (2010).

15. Standish, J., Sheehy, M. & Warner, R. Use of otolith natal elemental signatures as natural tags to evaluate connectivity among open-coast fish populations. *Mar. Ecol. Prog. Ser.* **356,** 259–268 (2008).

16. Bradbury, I. R., Campana, S. E. & Bentzen, P. Estimating contemporary early life-history dispersal in an estuarine fish: integrating molecular and otolith elemental approaches. *Mol. Ecol.* **17,** 1438–1450 (2008).

17. Charrad, M., Ghazzali, N., Boiteau, V. & Niknafs, A. NbClust: An R package for determining the relevant number of clusters in a data set. *J. Stat. Softw.* **61,** 1–36 (2014).

18. Porras-Hurtado, L. *et al.* An overview of STRUCTURE: applications, parameter settings, and supporting software. *Front. Genet.* **4,** (2013).

19. Matta, M. E., Short, J., Helser, T., Ormseth, O. & Miller, J. Spatial and temporal variation in otolith elemental chemistry of young-of-year Pacific cod in the Gulf of Alaska.

20. Selkoe, K. A., Henzler, C. M. & Gaines, S. D. Seascape genetics and the spatial ecology of marine populations. *Fish Fish.* **9,** 363–377 (2008).

**Appendix**

Metadata

Scripts

All R scripts used for this study can be found in the “scripts” folder in the [Otolith Analyses directory](https://github.com/mfisher5/PCod-Korea-repo/tree/master/otolith_analyses) of Mary Fisher’s Github page. *For the final paper, I will have a README in this folder to describe the purpose of each script.*

Additionally, downloading the [Otolith Analyses directory](https://github.com/mfisher5/PCod-Korea-repo/tree/master/otolith_analyses) or branching [the PCod-Korea-repo](https://github.com/mfisher5/PCod-Korea-repo) will provide the file structure and data needed to replicate this study’s analyses in R.