Exxon Valdez Oil Spill
Restoration Project Final Report

Investigation of Pacific Herring (*Clupea pallasii*) Stock Structure in Alaska Using Otolith Microchemistry and Heart Tissue Fatty Acid Composition

Restoration Project 070769
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Edward O. Otis[1]
Ron Heintz[2]
Jacek Maselko[2]

[1]Alaska Department of Fish and Game- Division of Commercial Fisheries
3298 Douglas Place
Homer, AK 99603

[2]National Marine Fisheries Service-Auke Bay Laboratory
11305 Glacier Hwy.
Juneau, AK 99801

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Study History: A Detailed Project Description (DPD), "Temporal stability of fatty acids used to discriminate Pacific herring (*Clupea pallasii*) in Alaska", was submitted to the *Exxon Valdez* Oil Spill Trustee Council (EVOS-TC) in April 2004. Following review by the Trustee Council, Project 050769 was ultimately approved for FY05 funding. During March, April, May, and June 2005-2007, Pacific herring were collected at 14 sites from seven putative herring stocks around Alaska (e.g., Sitka, Prince William Sound, Kamishak, Kodiak, Togiak, and Kuskokwim Bay). Additional samples were collected from Dutch Harbor in July 2006 and Kodiak in December 2007. Hearts were removed from 1,744 herring and stored for subsequent fatty acid analysis. In August 2006, we submitted a revised DPD for additional FY07 funding to process otolith samples that were simultaneously collected from 1,010 herring sampled for fatty acid analysis in 2006. Virtually all of the herring samples used for this study were collected and field processed by Alaska Department of Fish and Game (ADF&G) staff. ADF&G technicians also determined the age, sex, and maturity of all sampled herring. Fatty acid analysis of heart tissue samples was conducted by personnel at the Auke Bay Lab (NMFS).

Abstract: Understanding the structure of Alaska's Pacific herring stocks is relevant to how these exploited populations should be assessed and managed. We evaluated the capability of heart tissue fatty acid profiles and otolith microchemistry to identify the stock of origin for herring sampled from fourteen spawning aggregations associated with seven putative herring stocks throughout Alaska (e.g., Sitka, Prince William Sound, Kamishak, Kodiak, Togiak, Kuskokwim Bay, and Dutch Harbor). Fatty acid profiles were determined by performing trans-esterification and fatty acid chromatography on purified lipids from whole hearts. Otolith microchemistries were measured (ppm) using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Across-year comparisons were made to evaluate the temporal stability of fatty acid biomarkers that successfully discriminated putative stocks at multiple spatial scales within-years. Cross-validation of discriminant functions and nMDS with ANOSIM revealed that considerable shifts in fatty acid composition occurred for most stocks across short (1 year) and long (4-5 year) time periods. Temporal shifts also made it difficult to identify stock of origin for a putative mixed-stock sample collected during winter. Otolith edge microchemistries were significantly different between some putative stocks at multiple spatial scales, but nMDS and ANOSIM suggested that this difference was infinitesimal. We used heart tissue fatty acid profiles to discriminate Alaskan herring stocks at relatively fine spatial scales when we built models from data collected within the same annual spawning period, but we were unsuccessful discriminating unknown samples (e.g., from mixed stock fisheries) collected outside the spawning period, or in other years.

Key Words: Bering Sea, *Clupea pallasii*, Dutch Harbor, elemental analysis, fatty acid, Gulf of Alaska, Kamishak, Kuskokwim, Kodiak, LA-ICPMS, mixed-stock fishery, otolith microchemistry, Pacific herring, Prince William Sound, Sitka, stock identification, stock structure, Togiak.

Project Data: *Description of data* - data collected during the course of FY05-08 field activities include: age, sex, and size statistics on 1,058 herring, otolith microchemistry (n = 283), and heart tissue fatty acid profiles (n = 592) from mature herring aged 2 to 15. Herring were collected during March-July 2005-2007 and November 2007 from focal spawning locations in southeast Alaska (two locations, total n = 254), PWS (two locations, total n = 201), Kamishak Bay (two locations, total n = 281), Kodiak Island (two locations, total n = 360), Togiak Bay (two locations, total n = 268), Bering Sea (two locations, total n = 225), and Dutch Harbor (one location, n = 30). Heart tissues (n = 592) that were analyzed for fatty acid profiles were necessarily destroyed during processing. However, we collected additional heart tissue samples from Sitka (155), PWS (177), Kamishak (167), Kodiak (217), Togiak (169), and Bering Sea (141) to archive in frozen storage (-70 C) at the Auke Bay Lab (NMFS). Unused otoliths from approximately 590 herring collected in these same six areas in 2006-7 are archived in frozen storage (still in heads) at the ADF&G office in Homer. Fin clips from approximately 873 herring collected in these six areas in 2006-2007 are archived in ethanol at the ADF&G office in Homer. All electronic data (e.g., age, sex, size, fatty acid and otolith microchemistry profiles) are maintained in an Excel spreadsheet at the ADF&G office in Homer. *Custodian* - Ted Otis, 3298 Douglas Place, Homer, Alaska 99603-8027, email: ted.otis@alaska.gov)

Citation: Otis, E.O., R. Heintz, and J. Maselko. 2010. Investigation of Pacific herring (*Clupea pallasii*) stock structure in Alaska using otolith microchemistry and heart tissue fatty acid composition**.** *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 070769), Alaska Department of Fish and Game, Division of Commercial Fisheries, Homer, Alaska.

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EXECUTIVE SUMMARY

Pacific herring (*Clupea pallasii*) are an important component of the marine ecosystem providing a trophic pathway for energy flowing from secondary producers to apex predators, including humans. Two related aspects of herring life history that remain poorly described are the degree to which herring return to natal areas to spawn and the scale at which population structure exists within large geographic areas. These key characteristics are directly relevant to how exploited herring stocks should be assessed and managed.

Many diverse techniques have been proposed to investigate population structure (Pawson and Jennings 1996; Begg et al. 1999a). Two of the more promising methods recently developed include fatty acid analysis (FAA) of select body tissues and elemental analysis (EA) of otoliths. Recent studies have suggested that the fatty acid composition of phospholipids in some body tissues (e.g., heart tissue, brain, eggs) have a stable genetic basis, indicating these tissues may be appropriate as stock identifiers (Joensen and Grahl-Nielsen 2000; Joensen et al. 2000, Kwetegyeka et al. 2006). Because otoliths are acellular, their chemical composition is stable (Campana and Neilson 1985) and can help identify subtle differences in aquatic environments fish are exposed to during their life (Gunn et al. 1992; Radtke and Shafer 1992; Secor 1992). This record of environmental exposure relies on the assumption that otolith composition is, in part, determined by the chemistry of the water occupied by the fish (Radtke and Shafer 1992; Campana and Gagne 1995).

In this study, we evaluated two innovative methods for discriminating herring stocks over broad (> 750 km) and fine (≤ 100 km) spatial scales- analysis of heart tissue fatty acids and otolith chemistry. Tools that can identify small scale differences among sub-populations are necessary to help state fishery managers develop appropriate strategies for assessing and managing putative herring stocks that exhibit varying degrees of interconnectivity (Waples and Gaggiotti 2006).

During 2005-2007, we sampled herring from fourteen spawning aggregations associated with seven putative herring stocks throughout Alaska (e.g., Sitka, Prince William Sound, Kamishak, Kodiak, Togiak, Kuskokwim Bay, and Dutch Harbor). Specifically, our objectives were to 1) assess the temporal stability and biological variability of stock discrimination criteria derived from fatty acid analysis of cardiac tissues; 2) assess whether the stock(s) of origin for herring harvested in winter food/fisheries can be determined by comparing their heart fatty acid composition to those of local area spawning aggregations; 3) using samples from the same individual fish, assess whether population sub-unit boundaries derived from otolith chemistry match those derived by fatty acid analysis; and 4) assess whether the stock(s) of origin for herring collected during fall/winter can be determined by comparing their otolith chemistry to those of local area spawning aggregations.

Fatty acid profiles were determined by performing trans-esterification and fatty acid chromatography on purified lipids from whole hearts. To assess biological variability, we collected herring randomly from each population sampled and compared our ability to correctly identify “unknown” samples to our pilot study results, where samples were controlled for age, sex, and maturity. Similar to our pilot study (Otis and Heintz 2003), we used discriminant function analysis (DFA) to compare samples at broad (e.g., across regions) and fine spatial scales

(e.g., among areas and individual sample sites within areas). The reliability of discriminant functions derived from fatty acids was examined by cross-validation using the leave-one-out method (Huberty 1994), with reliability calculated as the percentage of correct identifications. To assess the temporal stability of stock discrimination criteria derived from heart tissue fatty acid analysis, we developed models representing putative herring stocks using one year's sample data and evaluated our ability to correctly identify "unknown" samples collected from the same areas in other years. Because we resampled the same areas from our 2001 pilot study, we were able to evaluate both long (2001 to 2005/2006) and short term (2005 to 2006) temporal stability. We also included a spatial component to our analyses to assess whether temporal stability was similar across regions and areas. To assess our second objective, we collected herring during early winter in an area that traditionally has a fall/winter food/bait fishery and sought to identify the stock of origin from this putative mixed-stock sample based on DFA models built from data derived from local area spawning stocks.

We used laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) to measure otolith composition at two specific locations (core and edge) on each herring sampled for fatty acid analysis in 2006. The core analysis was intended to represent the larval rearing environment (MacDonald et al. 2008) and the edge analysis was assumed to represent the spawning location where mature adult herring were captured (Hamer and Jenkins 2007). We used the same statistical methods employed for FAA to analyze the otolith core and edge chemistries. Otoliths were collected in 2006 only, so the temporal stability of otolith chemistries was not evaluated in this project. To assess population sub-unit boundaries, we compared otolith edges and cores from herring collected at multiple spatial scales (e.g., region, area, site). We compared otolith chemistries from herring collected during fall/winter to those of local area spawning aggregations to evaluate whether we could determine the spawning stock of origin.

Our results suggest that fatty acid analysis of heart lipids was a reliable method for discriminating putative herring stocks at multiple spatial scales (region, area, site) corresponding to linear separations among sample centroids of > 750 km (region), 250-750 km (area), and sometimes even 75-250 km (sample sites), as long as samples were compared within and not across years. DFA correctly identified the region of origin 99%, 86%, and 90% of the time in 2001, 2005, and 2006, respectively. Analysis of similarity (ANOSIM) R statistics revealed significant differences among all regions compared, except southeast Alaska (SEAK) versus the northern Gulf of Alaska (NGA) in 2001. ANOSIM R statistics also revealed significant differences in fatty acid composition at the area scale, except for Kamishak Bay vs. Prince William Sound in 2005. DFA cross-validation success at the area scale varied among the areas sampled, ranging from 70-89%. When individual sample sites were compared within management areas, significant differences were sometimes found from samples collected just 75-250 km apart (e.g., Sitka Sound vs. Hoonah Sound; east [Kiliuda Bay] vs. west Kodiak [Uganik Bay]; and southern [Chenik/Nordyke] vs. northern Kamishak [Iniskin/Oil Bay]). In most cases, our *a priori* stock identities appeared to best describe the fatty acid data structure. Contrary to expectations, the Dutch Harbor sample, although unique, grouped closer to North Gulf of Alaska (NGA) than to Bering Sea herring.

We did not observe a high degree of temporal stability in fatty acid composition across years for most of the stocks sampled. Cross-validation of discriminant functions and non-parametric

multidimensional scaling (nMDS) with ANOSIM revealed considerable shifts in fatty acid composition for most stocks across both short (1 year) and long (4-5 year) time periods. The one notable exception was in southeast Alaska where both the Sitka Sound and Hoonah Sound samples exhibited considerable stability across sample years. The temporal shifts we observed in other populations made it difficult to identify stock of origin for the putative mixed-stock sample we collected during the winter. Second stage nMDS plots and ANOSIM R statistics comparing herring collected throughout the NGA indicated that the Kodiak Winter 2007 samples were significantly different from all NGA samples collected in 2005 and 2006, suggesting a dietary effect on fatty acid composition.

We found little evidence of stock structure when comparing the elemental composition of otoliths that were analyzed by LA-ICPMS. Very little separation was apparent among regions when we examined otolith cores, however, the North Gulf of Alaska (NGA) appeared to be significantly different from all other regions sampled when we compared otolith edges. Although statistically significant, the very small ANOSIM R values indicated little difference existed among regions, regardless of the zone of the otolith analyzed.

We conclude that heart tissue fatty acid profiles can be used to discriminate Alaskan herring stocks at relatively fine spatial scales when models are built from data collected within the same year, but they cannot be reliably used to discriminate unknown samples (e.g., from mixed stock fisheries) collected in other years. The failure of fatty acid analysis to be effective as a mixed-stock analysis tool for most stocks is disappointing; however, its value for discriminating fine scale stock structure over short time periods should not be undervalued. In the near term, fatty acid analysis may be useful towards further resolving a number of management questions regarding stock structure in Prince William Sound, Kodiak/Kamishak, Togiak/Dutch Harbor, and in southeastern Alaska. Also, if future work corroborates our finding that some stocks (e.g., Sitka Sound and Hoonah Sound) exhibit intra- and inter-annual temporal stability, then fatty acid analysis could be used as a basis for understanding the home ranges, over-wintering areas, and larval dispersal patterns for these stocks.

INTRODUCTION

Pacific herring (*Clupea pallasii*) are an important component of the marine ecosystem, providing a trophic pathway for energy flowing from secondary producers to apex predators. Throughout their life, herring are prey to birds (Logerwell and Hargreaves 1997), marine mammals (Iverson et al. 1997), invertebrates (e.g. hydromedusae: Wespestad and Moksness 1989), other fish (Tanasichuk et al. 1991), and humans (Fischer et al. 1997). The commercial sac roe herring fishery harvest in Alaska has averaged approximately 33 thousand tonnes over the past 10 years.

Despite several decades of study and over a hundred years of commercial exploitation targeting herring, considerable uncertainty continues to exist regarding: 1) the spatial scale at which population structure exists and, 2) the degree to which herring return to natal areas to spawn. These fundamental life history traits are directly relevant to how exploited herring stocks should be assessed and managed (Hourston 1982; Wheeler and Winters 1984; Hay and McCarter 1997; McQuinn 1997, Begg et al. 1999a). State fishery managers require a tool that can discriminate ecologically significant populations among adjacent spawning aggregations that are exploited during spring sac-roe herring fisheries. They also require a mixed stock analysis tool that allows them to investigate whether winter herring fisheries (e.g., food/bait fisheries) target only the local spawning stock or a mixture of nearby stocks that aggregate during winter. The ability to manage stocks discretely is a principal component of sustainable fisheries management- one that requires the ability to accurately apportion the catch from mixed stock fisheries (Hauser and Ward 1998, Begg et al. 1999a).

In this study, we were interested in identifying population structure of Pacific herring throughout Alaska, but most notably in the North Gulf of Alaska (NGA) and Prince William Sound (PWS). For instance, fisheries that target herring spawning in Kamishak Bay and the Northwest side of Kodiak Island are managed separately; however, there is reason to believe that fish from the two areas mix in northern Shelikof Strait as rearing juveniles and over-wintering adults (C. Burkey, Alaska Department of Fish and Game, personal communication). We were also interested in looking for evidence of population structuring within putative herring stocks (e.g., PWS, Togiak, Kamishak Bay) that contained temporally and spatially separated spawning aggregations. Previous efforts to identify population structure among PWS herring using microsatellite DNA variation found little evidence for genetic isolation among the four spawning aggregations sampled, with the possible exception of Port Chalmers (O'Connell et al. 1998).

Researchers have attempted to use many different techniques to distinguish among fish stocks, including: tagging studies (Hourston 1982, Hay et al 2001), nuclear, microsatellite, and mtDNA analysis (O'Connell et al. 1998, Small et al. 2005), enzyme electrophoresis (Schweigert and Withler 1990), multilocus genotype 'familyprinting' (Letcher and King 1999), parasite markers (MacKenzie and Abaunza 1998; Oliva and Ballón 2002), scale pattern analysis (Rowell 1981; Barros and Holst 1995), mass marking of otoliths using temperature manipulation (Hagen et al. 1995; Joyce et al. 1996; Courtney et al. 2000) and fluorescent markers (Beckman et al. 1990), stable isotope composition of otoliths (Gao et al. 2001, Ayvazian et al. 2004), otolith microstructure (Brophy and Danilowicz 2002, Clausen et al. 2005, Dickey-Collas et al. 2005), otolith microchemistry (Thresher 1999, Otis and Heintz 2003), fatty acid analysis (Grahl-Nielsen

and Ulvund 1990, Otis and Heintz 2003) and the use of life history (Begg et al. 1999b), phenotypic (Swain and Foote 1999), meristic and morphometric characteristics (Schweigert 1990).

Many traditional stock identification techniques have proven to be inconclusive on marine forage fish, particularly at fine spatial scales. For example, O'Connell et al. (1998) found that herring from Prince William Sound (PWS) and the Bering Sea were genetically divergent, but they were unable to find similar divergence among stocks sampled within the north Gulf of Alaska (GOA). Similarly, Beacham et al. (2002), and also Small et al. (2005), found very little microsatellite variation in herring along the British Columbia coast and Puget Sound, respectively, except where differences in spawning timing were great enough to effectively isolate select spawning aggregations. Bentzen (1998) found that the magnitude of genetic variation observed among sampling years within locations was equal to or greater than the variation observed among sea basins for Gulf of Alaska and Bering Sea herring. Bentzen (1998) concluded that detectable genetic separation of spawning aggregates from the same race (e.g., GOA) may not be possible on spatial scales less than 1,000 km, unless spawning timing is substantially distinct.

The difficulty encountered with genetic markers is likely due to the relatively high stray rates exhibited by herring (e.g., Tester 1949; Cushing and Burd 1957; Hourston 1982; Wheeler and Winters 1984, Hay et al. 2001). The utility of allozyme markers for discriminating among putative stocks can be compromised by very little gene flow between populations (Smith and Jamieson 1986; Bembo et al. 1996; Hauser and Ward 1998, Waples 1998). In particular, Waples (1998) observed that "because the amount of migration necessary to obscure most genetic evidence of stock structure (only a handful of individuals per generation) is generally inconsequential as a force for rebuilding depleted populations on a time scale of interest to humans, there is no guarantee that genetic methods alone will provide sufficient precision for key management decisions involving marine species". Indeed, a number of researchers have recently emphasized the need to supplement genetics with a more holistic approach to stock discrimination of marine pelagic fish (Hauser and Ward 1998, Waldman 1999, Gustafson 2005, Hatfield et al. 2005, Cadrin et al. 2005).

In the absence of more definitive tools, many fishery managers have traditionally used spawning timing and location as proxies to roughly define herring stock structure. The logical assumption is, the greater the temporal and spatial separation between spawning aggregates, the greater the likelihood that they are discrete stocks. However, problems can arise when mixing of putative stocks occurs across jurisdictional boundaries. Ware and Tovey (2004) provide a summary of spawn disappearance and recolonization events that occurred along the British Columbia coast from 1943-2002. Anecdotal observers have also reported examples in which the abundance of one presumptive spawning stock "crashes" while an adjacent area's presumptive stock simultaneously increases by a commensurate amount. Such observations of "spawner relocation" highlight the behavioral complexity of herring (Overholtz 2002; Hay and McKinnell 2002; Huse et al. 2002, Ruzzante et al. 2006, Mitchell 2006) and raise questions regarding stock discreteness and population "sub-units" (Stephenson 1999).

Answering the question of stock discreteness is likely to be the key to developing management programs that sustain forage species. Stephenson (1999) states "…there has been little attention

paid recently to the complexity of spawning components within management units. Several marine finfish species appear to have more complex stock structure than is recognized, and in many cases, management units contain stock complexes or metapopulations with several spawning components rather than single discrete populations. Unfortunately, these spawning components are typically difficult to define from traditional fisheries data, or to discriminate by conventional stock identification techniques". Stephenson (1999), and this report hereafter, uses the term population "sub-unit" to characterize temporally/spatially dispersed spawning components within larger, traditionally recognized stocks. Stephenson (1999) warned that "failure to recognize or to account for this complex stock structure in management may lead to erosion of spawning components, with unknown ecological consequences". Preservation of biocomplexity (e.g., diverse spawning timing and behavior) has been cited as a key ingredient for maintaining the sustainability of exploited fish populations (Ruzzante et al. 2006) and for maintaining resilience to changes in environmental conditions (Hilborn et al. 2003, Secor 2007).

Given the challenges associated with using traditional genetic stock identification techniques to determine stock structure for some marine species, several authors have recommended a multi-disciplinary approach (Begg and Waldman 1999, Hatfield et al. 2005, Cadrin et al. 2005) that includes comparative studies specifically designed to contrast results from alternative methods applied to the same sample sets (Waldman 1999), however, surprisingly few published studies have attempted to do so (but see Miller et al. 2005 and Jonsdottir et al. 2006). This project sought to provide a comparison of two "cutting edge" technologies that have recently been successfully applied to stock identification of marine fish- heart tissue fatty acid analysis and otolith microchemistry.

During the pilot study (Otis and Heintz 2003) that preceded the current work, we used heart tissue fatty acid profiles to discriminate known herring stocks and revealed differences among putative stocks at relatively fine spatial scales ($\geq$ 100 km). Because samples were collected in only one year and we controlled for age, sex, and maturity, the present study was designed to evaluate the temporal stability of the biomarkers we identified and also to determine if the success of our pilot study could be repeated without controlling for age and sex.

Fatty acid analysis (FAA) has been used successfully to discriminate among populations of several marine species (e.g. Grahl-Nielsen and Mjaavatten 1992, Castell et al. 1995, Pickova et al. 1997, Joensen et. al. 2000), including herring (Grahl-Nielsen and Ulvund 1990). Published studies indicate that the fatty acid composition of cardiac tissues are the least influenced by environmental factors (Viga and Grahl-Nielsen 1990), and are the most sensitive at discriminating stocks over small geographic scales (Grahl-Nielsen and Mjaavatten 1992).

Whether or not detection of stock-specific discernable differences in phenotypic traits (e.g., fatty acid composition and otolith chemistry) is evidence of population structure is open to debate (Waples 1998, Begg et al. 1999a, Begg and Waldman 1999). That debate has particular relevance to this project since many studies have shown that the fatty acid compositions of some tissues and lipid classes are highly sensitive to changes in diet and the environment (e.g., Hazel 1984, Henderson and Tocher 1987, Kirsch et al. 1998, Cordier et al. 2002, Turner and Rooker 2005). Therefore, demonstrating that the variation in heart tissue fatty acid composition

observed between stocks exceeds that imposed by the environment on a given stock will be a key element in the development of this method (Begg et al. 1999a).

Our pilot study (Otis and Heintz 2003) had limited success discriminating herring stocks at fine spatial scales using otolith chemistry as measured by an electron microprobe. However, several studies have reported greater success using more precise instruments to measure trace elements in otoliths, so we also included an otolith component to the present study, for comparative purposes. Otoliths (fish ear stones) are composed of $CaCO_3$ in the form of aragonite. The otolith forms through concentric additions of mineralized tissue around a central nucleus, or primordium, with daily additions during the larval and early juvenile stages of life (Pannella 1980, Campana 1999). Otoliths are acellular, so once accreted, the material is not resorbed or reworked to any significant degree (Campana and Nielson 1985). The microchemistry of the otolith, as well as the physical banding patterns, provide unique insights into the environmental history of the fish but also provide information about major physiological stresses such as mating, low winter temperatures, and starvation (Berghahn and Karakiri 1990, Metcalfe et al. 1992, Smith 1992, Zhang and Runham 1992, Molony 1996). The minor and trace element composition of otoliths may allow for the reconstruction of the composition of aquatic systems in which the fish lived (Kalish 1991, Gunn et al. 1992, Radtke and Shafer 1992, Secor 1992, Campana 1999, Kennedy *et al.* 2002, Wells et. al. 2003).

The use of otoliths as records of environmental exposure is based on the premise that otolith microchemistry reflects differences in water chemistry in the environment (Radtke and Shafer 1992, Campana and Gagne 1995). The trace elemental composition of fish otoliths is determined by the elemental composition of the endolymph (Kalish 1989, 1991). The concentration of various trace elements in the environment and the physiology of the fish largely determine the composition of the endolymph. Physiological processes may be modified by temperature (Kalish 1991), or subtle differences in the genetics of the fish affecting the uptake of various elements and their inclusion in the endolymph (Thresher et al. 1994).

Otolith microchemistry has been used to identify stocks of pink snapper, (Edmonds et al. 1989), orange roughy (Edmonds et al. 1991), yellow-eye mullet (Edmonds et al. 1992), Atlantic cod (Campana and Gagne 1995, Campana et al. 1995), walleye (Bickford and Hannigan 1990), salmonids (Kalish 1990), Icelandic cod (Jonsdottir et al. 2006), and walleye pollock (FitzGerald et al. 2003), among others. Thresher (1999) provides a comprehensive review of the use of otolith elemental composition as stock discriminators and offers some cautionary suggestions for researchers interested in employing this promising technique.

Successful application of otolith elemental analysis for stock discrimination is likely dependent on the extent of the differences in water chemistry between the environments inhabited by each stock and the precision of the instruments used to measure trace elements. Laser ablation-inductively coupled plasma- mass spectrometry (LA-ICPMS) can be used to analyze trace elements (parts per million) at relatively specific loci (25-30 μm) on the otolith (Gray 1985, Denoyer et al. 1991). Electron microprobes (EM) allow analysis of very specific loci (e.g., 4 μm; FitzGerald et al. 2004), albeit at reduced resolution (parts per thousand; FitzGerald et al. 2004). Techniques that target specific loci, such as EM and LA-ICPMS, are most appropriate for

identifying stocks that spawn in different environments but later reside in similar environments (Coutant and Chen 1993).

In this study, we evaluated the capabilities of heart tissue fatty acid composition and otolith microchemistry to identify the stock of origin for herring sampled from focal spawning aggregations in Southeast Alaska (SEAK; e.g., Sitka), the North Gulf of Alaska (NGA; e.g., Prince William Sound [PWS], Kamishak, Kodiak), the Bering Sea (e.g., Togiak, Kuskokwim Bay), and the Aleutian Islands (e.g., Dutch Harbor). This study addressed six principal research questions: 1) Does herring heart tissue fatty acid composition vary significantly among putative spawning stocks?; 2) Are stock-related differences in fatty acid composition temporally stable?; 3) Does herring heart tissue fatty acid composition provide discriminatory power to correctly assign fish to their collection location?; 4) Does herring otolith microchemistry vary significantly among putative spawning stocks?; 5) Does herring otolith chemistry provide discriminatory power to correctly assign fish to their collection location?; and 6) Are stock structure designations generated by fatty acid analysis and otolith elemental analysis complimentary? We also attempted to determine if herring collected from a potentially mixed-stock fishery during winter (Kodiak Food/Bait fishery) could be assigned to their stock of origin based on heart tissue fatty acid composition or otolith microchemistry.

Fatty acid profiles were determined by performing trans-esterification and fatty acid chromatography on purified lipids from whole hearts. Cross validation of discriminant function analysis (DFA) models we developed to evaluate within-year differences in fatty acid compositions were able to identify known samples to their correct region, area, and sometimes sample site of origin with reasonably good success (generally > 85% accuracy). These results are similar to those of our pilot study (Otis and Heintz 2003), despite the fact that we did not control for sex, age, or maturity in this study, demonstrating that the biological variability of heart tissue fatty acid profiles within populations is not greater than that exhibited across populations. Unfortunately, when we compared fatty acid profiles across years, we found very little temporal stability at any spatial scale, with the notable exception of samples from Southeast Alaska (e.g., Sitka Sound and Hoonah Sound). Probably due to the lack of temporal stability in fatty acid profiles, we also were unable to reliably assign winter Kodiak samples to their stock of origin based on DFA models built from samples collected from adjacent spawning stocks one and two year's prior. We concluded that heart tissue fatty acid profiles can be used to discriminate among Alaskan herring stocks at relatively fine spatial scales when models are built from data collected within the same year, but these models cannot be reliably used to discriminate unknown samples (e.g., from mixed stock fisheries) collected in other years.

We used laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) to determine the Sr:Ca and Mg:Ca ratios at two specific locations (core and edge) on the otoliths of each herring sampled for fatty acid analysis. The same statistical methods employed for FAA were used to look for spatial structure in the otolith data. Very little structure was apparent among regions when we examined otolith cores. Discriminant functions were only slightly better than random at identifying the sampling region for an unknown otolith. In contrast, the North Gulf of Alaska (NGA) appeared to be significantly different from all other regions sampled when we compared otolith edges. However, this statistically significant result was not corroborated by the

small ANOSIM R values and nMDS plots, which indicated very little difference existed among regions, regardless of the zone of the otolith analyzed.

OBJECTIVES

Fatty acid analysis

During the pilot study (Otis and Heintz 2003) that preceded the current work, we used heart tissue fatty acid profiles to discriminate known herring stocks and reveal differences among putative stocks at relatively fine spatial scales ($\geq$ 100 km). Because samples were collected in only one year and we controlled for age, sex, and maturity, the present study was designed to evaluate the temporal stability of the biomarkers we identified and also to determine if the success of our pilot study could be repeated without controlling for age and sex.

The original detailed project description (DPD) we submitted in April 2004 (050769) to evaluate the temporal stability of fatty acids listed the following objectives for FY05 - FY07 activities:

Objective 1) Assess the temporal stability and biological variability of stock discrimination criteria derived from fatty acid analysis of cardiac tissues.

Objective 2) Assess whether the stock(s) of origin for herring harvested in winter food/fisheries can be determined by comparing their heart fatty acid composition to those of local area spawning aggregations.

To assess the biological variability of fatty acids, we collected herring randomly from each population sampled and compared our ability to correctly identify known samples to our pilot study results, where samples were controlled for age, sex, and maturity. Similar to our pilot study, we looked for unique fatty acid groupings at broad (e.g., across regions) and fine spatial scales (e.g., among areas and even individual sample sites within areas). Results from these analyses are presented under the heading "*Reliability of identifying groups within years*".

To assess the temporal stability of stock discrimination criteria derived from heart tissue fatty acid analysis, we developed models representing putative herring stocks using one years sample data and evaluated our ability to correctly identify known samples collected from the same areas in other years. Because we resampled the same areas from our 2001 pilot study, we were able to evaluate both long (2001 to 2005/2006) and short term (2005 to 2006) temporal stability. We also included a spatial component to our analyses to assess whether temporal stability was similar across regions and areas. Results from these analyses are presented under the heading "*Reliability of identifying groups across years*".

To assess our second objective, we collected herring during early winter in an area that traditionally has a fall/winter food/bait fishery and sought to identify their stock of origin based on DFA models built from data derived from local area spawning stocks. Results from these analyses are presented under the heading "*Reliability of identifying stock of origin from winter samples*".

Otolith microchemistry

The goal of this component of our project was to determine whether otolith microchemistry was useful for discriminating population sub-units within traditionally recognized stocks of Pacific herring in Alaska (e.g., Sitka, PWS, Kamishak, Kodiak, Togiak, Dutch Harbor, Bering Sea). The pilot study that preceded this project (Otis and Heintz 2003) had limited success discriminating herring stocks at fine spatial scales using otolith chemistry as measured by an electron microprobe. However, several studies have reported greater success using more precise instruments to measure trace elements in otoliths of marine fish (e.g., FitzGerald et al. 2004, Jonsdottir et al. 2006). Because we collected otolith samples from the same individual fish used to evaluate the temporal stability of fatty acid compositions, this project offers the unique opportunity to directly compare two cutting edge herring stock identification techniques.

The DPD we submitted in August 2006 to add an otolith microchemistry component to our herring stock identification project (070769) listed the following objectives for FY07 activities:

Objective 3) Using samples from the same individual fish, assess whether population sub-unit boundaries derived from otolith chemistry match those derived by fatty acid analysis.

Objective 4) Assess whether the stock(s) of origin for herring collected during fall/winter can be determined by comparing their otolith chemistry to those of local area spawning aggregations.

The same statistical methods and spatial groupings employed for FAA were used to look for spatial structure in the otolith data.

METHODS

Study Area

Our study area included sampling locations extending from Sitka Sound (~57º N Latitude, 136º W Longitude), north to Prince William Sound (~61º N Latitude, 147 º W Longitude) and Kuskokwim Bay (~61º N Latitude, 165 º W Longitude), and west to Dutch Harbor (~54 º N Latitude, 166 º W Longitude; Figure 1). Except for Dutch Harbor and Togiak and Kuskokwim bays, which are in the Bering Sea, all sampling locations were within the Gulf of Alaska (GOA), with most of the samples coming from locations in the Northern Gulf of Alaska (NGA; Table 1). Two of the GOA's largest embayments provided three of our twelve sampling locations, two in Kamishak Bay in Cook Inlet, and one in Prince William Sound, while one of its largest island complexes, Kodiak/Afognak, provided two more of our sample locations.

The coastal bathymetry of the GOA is characterized by a broad continental shelf, except in Southeast Alaska where the shelf narrows and deep fjords abound. The physical, chemical, and biological characteristics of the coastal environment throughout our study area are influenced by the Alaska Coastal Current (ACC). The ACC originates in Southeast Alaska and follows the coast north across the entrances to PWS and Cook Inlet, past Kodiak and Afognak Islands via Shelikof Strait, around the Alaska Peninsula via Unimak Pass, and into the Bering and Beaufort

Seas. The ACC is characterized by low salinity due largely to the tremendous volume of freshwater flowing into it from the hundreds of glaciers and waterways found throughout its length. Pacific herring can be found spawning at many locations along Alaska's ubiquitous coastline with commercially viable populations of interest to this study being located in Sitka Sound, Prince William Sound, Kamishak Bay (Lower Cook Inlet), Kodiak/Afognak Island, and the Bering Sea (Dutch Harbor, Togiak and Kuskokwim bays).

Sample collection and storage

Mature Pacific herring were collected from thirteen focal spawning locations within seven historically important herring spawning areas in Alaska. Unlike our pilot study (Otis and Heintz 2003), we did not control samples in the present study for age, sex, and maturity so our results represent the natural variability inherent in the populations sampled.

In 2005, we generally attempted to collect 75 heart tissue samples from each of two temporally/spatially separated spawning aggregations in each area. In 2006, we attempted to repeat our heart tissue sample collections at the same sites, while also collecting otoliths and fin clips for future analyses. If fish were not available at a target location in a given year, or if samples were destroyed during shipping, replacement samples were collected from an alternative site nearby whenever possible. Shipment of hazardous materials such as liquid nitrogen has become much more problematic in recent years. This has necessitated the use of "dryshippers", which have a much shorter "shelf-life" for maintaining sample preservation. On two occasions, samples shipped from remote areas were not viable because they arrived at the lab thawed. Approximately 30 herring from each viable sample event were randomly selected and processed for fatty acid and otolith analysis. The balance of the heart tissue samples were archived in frozen storage (–70° C). Unused otoliths were stored frozen inside herring heads and fin clips were archived in individually labeled vials of ethanol.

For each specimen, length (standard [SL] and/or fork [FL] length in cm), sex, and gonad maturity was determined and a scale was removed to determine the age of the fish. Heads were removed and stored frozen in individually labeled plastic bags for later laboratory processing of the otoliths. Whole hearts were removed, transferred to labeled vials, placed in liquid nitrogen, and stored at –70° C until analyzed (Ackman et al. 1969; Grahl-Nielsen and Mjaavatten 1992).

Fatty acid analysis

Samples collected in the field and shipped in liquid nitrogen to the Auke Bay Lab (ABL) were processed to determine their fatty acid compositions. A set of 30 individuals was randomly selected for processing from each of the sample collections. Only samples for which we had reliable ages were eligible for selection. The samples selected for processing were then randomly assigned to batches for processing in the chemistry laboratory. Batches consisted of approximately 27 samples plus quality control samples.

Lipids were extracted from whole herring hearts using a vacuum membrane filtration with isopropanol. Hearts were initially minced and combined with 3.5 ml SafTest® STD Prep Reagent (MP Biomedicals, Solon, OH), consisting of isopropanol with proprietary antioxidant additives. Samples were vortexed for 1 minute and incubated at 45˚C for 15 min. Extraction mixtures were poured into membrane filtration units and filtered via vacuum at approx. 254 mm-Hg until all

visible solvent had passed through the filter. The extracted lipid solution was re-incubated at 45˚C, and a 5 µl aliquot was removed for transesterification. Transesterification of extracted lipids converted heart fatty acids into fatty acid methyl esters (FAMEs). Lipid aliquots were initially spiked with a fatty acid internal standard (19:0) in hexane solvent and subjected to a Hilditch transesterification reaction, followed by saline and potassium bicarbonate washes (Christie 2003). The extracts were passed through a column of sodium sulfate and reduced in volume to less than 1 ml under vacuum. A recovery standard (21:0) was added before performing GC analysis.

FAMEs were analyzed by gas chromatography mass spectrometry. Spiked FAME samples were injected onto a Varian CP3800 gas chromatograph equipped with a 60m Varian Factor-4 VF23ms cyanopropyl-bonded fused silica column under a programmed temperature ramp from 50-250˚C. Chromatographically- separated FAMEs were detected with a Varian Saturn model 2200 mass spectrometer operating in selective ion storage mode. Varian Workstation 6.40 software was used to process raw data and quantify the chromatographic spectrum. Fatty acid concentrations were determined from six-point calibration curves developed for each FAME.

All analyses were performed in batches of 15-20 samples and each analysis batch included quality control samples. Quality control samples included reference standards with known lipid/fatty acid contents such as National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1946. Selected duplicate samples were analyzed to determine reproducibility and method blanks containing no sample material were included as quality control samples. Quality control analysis results were compared to established quality thresholds to ensure accuracy, precision, and analysis batch comparability. Samples below thresholds were reanalyzed. Coefficients of variation for duplicate analyses within a batch were generally less than 10%.

Otolith preparation and elemental analysis

Left and right sagittal otoliths were dissected from each specimen, cleaned with 0.5% bleach and 0.7% dechlor (sodium thiosulfate), and then placed in cell culture trays. The otoliths selected for elemental analysis were mounted proximal side up on petrographic glass slides using thermal plastic cement. Two otoliths were mounted on each slide. Placing the proximal side up ensured that the otolith center was close to the surface and could be exposed with gentle hand polishing using 9 and 3 micron Al_2O_3 lapping paper. During preparation, otoliths were viewed with both bright field and transmitted light microscopy to determine when fine scale patterns indicative of daily growth ring formation during the larval period were exposed to the surface. Once polishing was complete, the otoliths were then cleaned with Formula 409®, rinsed in deionized water, and air dried. The prepared samples were labeled and shipped to the Advanced Instrumentation Laboratory at the University of Alaska Fairbanks.

The slide mounted otoliths were placed into the sample chamber of a New Wave UP-213 Laser ablation system connected to an Agilent 7500ce ICP-MS. The laser was set up to ablate the selected transect moving at 10 microns/sec, focused to a depth of 5 microns, a pulse rate of 10Hz, a 25 micron spot size, and with the laser power set to 80%. Data collection was preceded by a thirty second laser warm-up period and a preablation pass with the same settings, except at

40% power, and 100 micron spot size. Two target areas were chosen for elemental analysis- the otolith "core" and the otolith edge. The core transect was curvilinear and followed a single growth ring in the early growth zone approximately 100-200 microns, radial distance, from the primordium. This region of the otolith was a common location for observing growth ring formation that likely corresponded with early feeding and larval development after the yolk sac had been completely absorbed, which occurs approximately 5-7 d post-hatch in Pacific herring. Our core target area likely did not corresponded with the first days of post-hatch, given the fact that larval herring do not lay down daily growth rings consistently until they are 50-60 days old (Campana et al. 1987). We chose this location over sampling a core region that included the primordium because several recent studies have reported elevated Mn levels within the primordium that may represent maternal or other non-environmental influences (Brophy et al. 2004, Ruttenberg et al. 2005, MacDonald et al. 2008). Our edge transect was also curvilinear and followed the outermost margin of the rostrum, the area of greatest growth on the otolith and therefore the area most likely to represent the capture location (Hamer and Jenkins 2007). The data from each transect was averaged, neglecting the signal shoulders. Data was collected from Mg^{24}, Ca^{42}, Ca^{43}, Mn^{55}, Sr^{86}, Sr^{87}, Sr^{88}, Ba^{137}, and Ba^{138}. Mn and Ba data were found to be below the limits of quantification for this study. Ca^{43} was used as an internal standard. External standards used were NIST-610 and FEBS-1.

Statistical analysis

A nomenclature is necessary to describe the various spatial comparisons we made. Samples were grouped at three spatial scales. From smallest to largest scale, these strata are hereafter referred to as: *site*, *area*, and *region*. Sites represent the actual location that samples were collected from individual spawning aggregations. Areas typically represent state-defined herring management areas (e.g., Sitka, Prince William Sound, Kamishak, Kodiak, Bristol Bay, Kuskokwim, and Dutch Harbor), within which more than one major spawning aggregation may be found. Regions represent the largest strata (e.g., Southeast Alaska [SEAK], North Gulf of Alaska [NGA], Bering Sea, Aleutian Islands) and often include more than one area (Figure 1; Table 1). Note that in cases where few sites were sampled, regions are synonymous with areas (e.g., Sitka was the only area sampled in Southeast) and sometimes sites (e.g., Iliulink Bay was the only *site* sampled in the Dutch Harbor *area* of the Aleutian Islands *region*; Table 1).

Fatty acid analysis

We used two spatially organized batteries of tests to examine the temporal stability of the fatty acid compositions. In the first battery of tests, we attempted to repeat the analysis performed in our 2001 pilot study. The tests were designed to indicate whether herring could be accurately assigned to their collection region/area/site in a given year based on fatty acid composition. These tests were based on the assumption that if herring cannot be accurately identified in a given year, then identification across years is less likely. The tests proceeded from broad to progressively finer spatial scales. First we evaluated the reliability of assigning unknown herring to their region of origin. Next we evaluated the reliability of correctly identifying herring from areas within a region. Finally, we evaluated the reliability of identifying herring from different sample sites within an area.

To evaluate the temporal and spatial variability of heart fatty acids used to discriminate herring stocks, our second battery of tests focused on determining if models derived from samples

collected in one year could be used to identify fish collected in another year. We proceeded from evaluating samples representative of broad spatial scales to progressively finer scales. In addition, we examined two temporal scales. To evaluate broad temporal scales, we used models derived from fish sampled in 2001 to identify individuals collected in 2005 and 2006. To evaluate fine temporal scales, we used models derived from fish collected in 2005 to identify fish sampled in 2006. Finally, to address our second objective, we attempted to identify fish collected from a food/bait fishery site using models developed from nearby spawning aggregates collected in 2005 and 2006. These fish were collected from Uganik Bay on Kodiak Island in late November 2007.

We used discriminant function analysis (DFA) for both batteries of tests. For the first battery of tests, the reliability of discriminant functions derived from fatty acids was examined by cross-validation using the leave-one-out method. Reliability was calculated as the percentage of correct identifications. For the second battery of tests, we constructed discriminant functions from fish sampled in one year and then tested the reliability of correctly assigning individuals collected from another year. Reliability was calculated as the percentage of correct identifications and is reported as confusion matrices in the results. We used XLSTAT ver. 2009 (*www.xlstat.com*), an add-in for MS Excel, for the DFA analysis.

One undesirable feature of DFA is that the results are dependant on the number of groups used in estimating the functions (McGarigal et al. 2000). Each time a new location is added, the functions change. Consequently we also used non-parametric multidimensional scaling (nMDS) with analysis of similarity (ANOSIM) to evaluate the discriminating power of fatty acids. All analyses were performed using Primer-E version 6 (Clarke and Warwick 2001). nMDS with ANOSIM is an iterative space-reducing procedure used to examine spatial relationships between multivariate observations (McGarigal et al. 2000). For the first battery of tests, our expectation was that fish from different geographic areas would map to different locations in the nMDS plots unless they had indistinguishable fatty acid compositions. For the second battery of tests, our expectation was that samples collected from a given location would occupy the same area in nMDS plots regardless of year they were sampled. The basis for these expectations is explained in more detail below.

nMDS with ANOSIM theory and application

nMDS reduces multivariate observations into a number of dimensions that can be viewed graphically with minimal distortion of the spatial relationships between observations. The spatial relationships are determined from a matrix of distance measures calculated between each pair of observations in the data set. Consequently, the results of nMDS are independent of the number of samples or groups in the set. Samples located near each other in nMDS plots can be construed as being more similar than those located farther apart. The distance matrix used for our analysis was calculated from the Euclidian distance between each pair of observations.

ANOSIM provides a mechanism for determining if the arrangement of observations in the nMDS plot differs from random. ANOSIM produces the statistic R, which reflects the distance between a given pair of observations or pair of groups. Values of R can range between -1 and 1, but typically fall between 0 and 1. High R values indicate two individual observations or groups of individuals are different from each other. An R value of 0 indicates that the group structure is

not different from a random arrangement. If values fall below 0, then there is greater variability within a group than between the groups examined. When groups of observations are compared, R can be construed as the centroid distance between the groups. Because R is a distance measure, an nMDS analysis can be performed on a matrix describing all the pair-wise differences between groups in a data set. This nMDS performed on a matrix of R values is referred to as a second-stage nMDS.

Pre-processing of fatty acid data

We transformed the fatty acid compositions prior to constructing the discriminant functions and distance matrices needed for our analyses. For the DFA we transformed the relative concentrations of the fatty acids by normalizing them to the concentration of 16:0 and taking the natural logarithm of the ratio. For the nMDS with ANOSIM we used the natural logarithm of the relative concentrations ($ln(x+1)$). Subsets of the fatty acid were used to reduce the number of predictor variables to less than the number of observations. Prior to transforming them, fatty acids were normalized to unit sum.

The set of fatty acids used for the two batteries of tests differed. The fatty acid analyses reported in our pilot study were performed by a different laboratory than the one that analyzed the 2005-2007 samples. These labs provided different sets of fatty acids. In 2001, we chose the fatty acids that comprised the bulk of the lipid mass recovered from the gas chromatograph (Otis and Heintz 2003). These fatty acids were used for the first battery of tests because we were attempting to replicate our pilot study results. However, we determined that a different set of fatty acids maximized our ability to detect temporal stability. For analyses aimed at assessing temporal stability we chose a set of fatty acids based on the reliability with which their signals from the gas chromatograph could be integrated. Peaks with shoulders and doublets were removed from consideration in order to reduce variability in the estimated concentrations.

Otolith Microchemistry

Comparisons of otolith chemical compositions were made on progressively finer spatial scales. Following the nomenclature introduced for FAA we initially compared fish from different regions. Initially, we compared otolith cores based on the assumption that cores represented conditions in the natal habitat (Kalish 1991, Campana 1999). We then repeated all our analyses using edge compositions based on the assumption that these would reflect the environment at the time of collection (i.e. spawning). All analyses were constrained to those otoliths collected in 2006 to minimize temporal effects associated with the otolith edge analysis. As long as it was possible to discriminate stocks on a regional basis we proceeded to examine structure at progressively finer spatial scales (e.g., area and site). The same statistical methods and spatial groupings employed for FAA were used to look for spatial structure in the otolith data.

In order to examine the hypothesis that herring return to natal habitats to spawn we compared the chemical composition of otoliths at the core and edge for otoliths collected at each of ten sites. We presumed that core composition would be representative of the environment at hatch and the edge composition would represent the environment at the time sampling (i.e., spawning). We hypothesized that if herring return to natal habitats to spawn and those habitats have unique water characteristics then we would expect core compositions to differ among stocks but not to

differ from the composition at the edge of the otolith. We compared edges and cores within a site by nMDS. Our expectation was that that a map of core compositions for all the sites generated by nMDS would overlap completely with a similar map generated from edge chemistries.

Pre-processing of otolith microchemistry data
We transformed the elemental compositions prior to constructing the discriminant functions and distance matrices needed for our analyses. Concentrations of Mg^{24} Sr^{86} Sr^{87} and Sr^{88} were normalized to Ca^{42} prior to analysis. $Ba^{137 \text{ and } 138}$ and Mn^{55} were also measured but observed concentrations were often below limits of quantification and these elements were deleted from the analysis.

RESULTS

Sample Collection

The total number of sample tissues collected and processed from each area is shown in Tables 1 and 2, respectively. The size and mean age of fish that were randomly selected for otolith and fatty acid analysis is shown in Table 3. Age-frequency distributions by sample area for fish randomly selected for otolith and fatty acid analysis are presented in Table 4.

Fatty Acid Analysis

Fatty acids recovered during this study were compared with those recovered from the same material using the process employed in our 2001 pilot study (Otis and Heintz 2003) and found not to differ (Table 5).

Reliability of identifying groups within years

Regional Differences
NGA and SEAK herring were consistently closer in fatty acid composition than either was to herring from the Bering Sea. DFAs indicated that the region of origin for herring collected in a given year could be reliably predicted (Table 6). During our 2001 pilot study, the region of origin was correctly identified 98.8% (94.4-100%) of the time using DFA (Otis and Heintz 2003). In the present study, herring were correctly identified to region 86.2% (83.3-89.6%) of the time in 2005 and 90.3% (84.2-100%) of the time in 2006. Generally, the greatest percentage of errors resulted from confusing NGA and SEAK herring. The propensity for NGA and SEAK to overlap was also demonstrated in the nMDS with ANOSIM. In 2001, the fatty acid compositions of the NGA and SEAK fish were most similar and both these groups were equally different from the Bering Sea (Table 7). The same relationships were observed in 2005. In 2006 the Aleutian Islands were included as a fourth region. Aleutian Islands fish were found to be more similar in composition to those from NGA and SEAK than the Bering Sea.

Differences across areas within regions

North Gulf of Alaska

The reliability of fatty acids as an indicator of the spawning location for NGA herring varied across years. The 2001 DFA correctly identified 88.6% of the herring sampled from Kamishak, Kodiak and Prince William Sound (Table 8). The DFA performed on fish in 2005 was less accurate. It correctly identified 70.4% of the herring sampled from Kamishak, Kodiak and Prince William Sound. The greatest number of errors was incurred in identifying Kamishak fish. Erroneously identified fish from Kamishak were identified as Kodiak or Prince William Sound with equal frequency. In 2006 no fish from PWS were sampled and the reliability of the DFA improved to levels observed in 2001. Overall, 81.2% of the fish sampled from Kodiak and Kamishak in 2006 were correctly identified (Table 8).

These annual changes in discreteness of the fatty acid compositions could also be observed using nMDS with ANOSIM. In 2001, the locations with the two most similar fatty acid compositions were Kodiak and Kamishak ($R = 0.173$; Table 9). In 2005, PWS and Kamishak were still the locations with the most similar compositions but the R statistics indicated Kamishak was also virtually indistinguishable from Kodiak. Kodiak was significantly different from Prince William Sound. This pattern was consistent with the results of the DFA. In 2006, differences in fatty acid composition re-appeared. The fatty acid composition of herring from Kamishak was significantly different from that of Kodiak ($R = 0.345$; $P < 0.001$).

Bering Sea

Areas could be differentiated by DFA with equally high reliability in 2005 and 2006. It was not possible to compare reliability in 2001 because only one area was sampled. In 2005, 82.3% of the herring could be correctly identified to their area of origin (Table 10). A similar level of reliability was observed in 2006 when 86.5% of the herring were correctly identified.

nMDS with ANOSIM also indicated that fatty acid compositions of fish from the different Bering Sea areas differed. In 2005, the distance between the Bristol and Kuskokwim Bay centroids was 0.239 ($P < 0.001$), nearly equal to that measured in 2006 0.249 ($P < 0.001$).

Southeast Alaska

Only one area was sampled in southeastern Alaska (Sitka), hence the reliability of identifying the area of origin could not be tested.

Aleutian Islands

Only one area was sampled in the Aleutian Islands (Dutch Harbor), hence the reliability of identifying the area of origin could not be tested.

Differences across sample sites within areas

The fatty acid composition of herring hearts from a given area diverged as the time elapsed between sampling events increased. In 2005 two sites were sampled from Sitka, Kodiak, Kamishak, and Kuskokwim and Bristol bays (Table 1). In 2006, two sites were sampled from Sitka, Kodiak, Kamishak, and Bristol Bay. The 2005 and 2006 Sitka sites and the 2005 Kuskokwim Bay sites were sampled approximately 30 days apart. The 2005 and 2006 Kodiak sites were sampled about 14 days apart, as were the 2005 Kamishak sites. The 2006 Kamishak

sites and all the Bristol Bay sites were collected within 3 days of one another. Comparisons between the two Sitka sites indicated significant differences in fatty acid composition in both years. The R statistics for comparisons between sites in the Sitka area were 0.831 ($P < 0.001$) in 2005 and 0.444 in 2006 ($P < 0.001$). DFAs indicated that the Sitka samples could be correctly identified more than 96% of the time (Table 11). Similarly, the Kuskokwim Bay sites differed in composition (R = 0.206 P < 0.001) and the DFA correctly identified 91% of the fish (Table 11). The Kodiak sites were also different in both of the years sampled (R = 0.284; P < 0.001). DFA correctly identified 91% of the Kodiak herring in 2005 and 90% in 2006. The Kamishak samples collected in 2005 also differed, but to a much lesser degree (R = 0.097; $P = 0.04$). In contrast, the northern and southern Kamishak samples (i.e., Chenik/Nordyke and Iniskin/Oil Bay) collected three days apart in 2006 were indistinguishable (R = 0.028; $P = 0.116$). A similar distinction between the 2005 and 2006 collections was observed in the Kamishak DFA's (Table 11). Overall, 76% of the fish collected in 2005 could be identified to the correct sample site, but only 55% could be correctly identified in 2006. Sites in Bristol Bay were always indistinguishable (R < 0.024; P > 0.096). Consequently, DFAs indicated that identification of samples from sites in Bristol Bay was no better than random (Table 11).

Reliability of identifying groups across years

Compositional differences between groups were stable over short time periods, and time ultimately accounted for the greatest amount of variation in fatty acid composition. We performed DFA's on herring collected from different regions and on areas within regions and on individual sample sites within areas. The fatty acid composition of herring collected in one year was used to identify herring to their appropriate stratum in the remaining years. Regardless of the geographic level investigated, the models constructed from the 2005 and 2006 collections were much better at identifying herring from the adjacent year than those collected in 2001. R statistics calculated for ANOSIMS provided quantitative measures in support of this trend. These results are presented and illustrated in more detail below.

Regional Differences

Herring could be reliably identified by region over short time periods, but they were indistinguishable over long time periods. DFA models constructed from fish collected in 2001 were unable to accurately identify fish collected in 2005 and 2006. The 2001 model correctly identified 43% of the fish collected from the Bering Sea, NGA or SEAK in 2005 or 2006 (Table 12). The 2006 Aleutian Islands samples had no counterpart in the 2001 data set and 97% of them were identified as NGA. The 2005 model was better at identifying samples collected in 2006 and 2007 than those collected in 2001. The 2005 model correctly identified 80% of the fish collected from the Bering Sea, SEAK, and NGA in 2006 and the NGA in 2007 (Table 12). Fish collected in 2006 from the Aleutian Islands were identified as NGA fish 83% of the time. Similarly, the 2006 model identified fish collected in 2005 and 2007 better than those collected in 2001. More than 69% of the fish collected in 2005 and 2007 were correctly identified. This relatively low percentage was due to errors assigning NGA fish to the Aleutian Islands. In contrast, the model only identified 35% of the fish collected in 2001 (Table 12).

nMDS with ANOSIM revealed further evidence of temporal shifts in fatty acid composition. The second-stage nMDS plot (Figure 2) indicated that time explains more of the variation in fatty acid composition than region. Within a year, the regional centroids had the lowest R statistics

and fish from NGA and SEAK were more similar to each other than either was to those from the Bering Sea (Table 13). The next lowest R statistics were observed between the adjacent years; 2005 and 2006. As the time elapsed between sampling events increased, the distance between a region's centroids also increased. Moreover, the spreading of regional centroids followed a consistent direction (Figure 2).

Differences across areas within regions

NGA

Predictive DFA models indicated there was little temporal stability in the fatty acid compositions in the fish collected from areas within the NGA. The DFA model constructed from fish collected in 2001 had little accuracy in identifying herring collected in 2005 and 2006. Fish collected in winter 2007 from Kodiak were identified as Kamishak fish 93.3% of the time by the 2001 model. Overall, the 2001 predictive model correctly identified 42.2% of the herring (Table 14). The predictive models constructed from the herring collected in 2005 and 2006 were no better. The 2005 model accurately identified 41.9% of the fish collected in 2001 and 2006. However, it was more accurate at identifying fish collected in 2006 than those from 2001. It correctly identified 67% of the fish collected from Kamishak and Kodiak in 2006 compared with 23.8% of the fish collected in 2001. It also identified the majority of the 2007 Kodiak fish as coming from PWS. The predictive model constructed from fish collected in 2006 accurately identified 32.1% of the fish collected in 2001 compared with 62% of those sampled in 2005. In addition it identified 70% of the 2007 Kodiak fish as coming from Kamishak.

The nMDS and ANOSIM indicated that time accounted for most of the variation in the fatty acid composition observed among herring in the NGA. Herring collected in 2001 occupy the lower left hand portion of Figure 3 (top panel), while those from 2006 and 2007 are found to the upper right; 2005 samples are intermediate. Examining individual sampling areas indicates that temporal shifts were larger at some locations than others.

This variation in the magnitude of temporal shifts was even more evident when nMDS plots of samples from different sites were compared (Figure 3, bottom panel). For example, the dissimilarity in fatty acid compositions for herring sampled at Nordyke Island in 2005 and 2006 was 0.362, while the dissimilarity for Uganik Bay over the same time period was 0.111. However, there was a clear separation in time between the samples collected in 2005 and 2006 when they were compared with those collected in 2001.

Bering Sea

Predictive DFA models constructed from herring sampled in the Bering Sea in 2005 and 2006 revealed little evidence of temporal stability. The predictive DFA model constructed from herring collected from the Bering Sea in 2005 reliably identified 100% the herring collected in 2001, but less than 65% of the herring collected in 2006 (Table 15). The predictive model constructed from herring collected in 2006 correctly identified 67% of the fish collected in 2001, and fewer than 67% of those collected in 2005.

The nMDS plot and ANOSIM indicated that time contributes significantly to the variation in fatty acid composition between areas and sample sites (Figure 4). Herring collected from areas in Bristol Bay in 2001 are located to the left of those collected in 2005. Those collected in 2006 are

located even further to the right (Figure 4, top panel). Distances between fish collected from the different areas in 2005 and 2006 are always the furthest from the herring collected from Bristol Bay in 2001.

Accounting for the variation in the fatty acid compositions of different samples collected from Bering Sea sites indicates the temporal variation in fatty acid composition is greater in some areas than others (Figure 4, bottom panel). Within areas, different sites were sampled in the different years (Table 1). The nMDS plot of the relative distances between these sample sites indicates that those collected in 2005 were more variable in fatty acid composition than those collected in 2006. Between 2005 and 2006 the shift in fatty acid composition for the Bristol Bay sample sites (i.e., Nunavachak and Hagemeister Island) was less than that for the Kuskokwim Bay sample sites (i.e., Toksook/Nelson Island and Goodnews Bay). Moreover, the shift in composition observed for the Nunavachak fish was much greater than that of the Hagemeister fish (Figure 4, bottom panel).

Southeast AK

The fatty acid compositions of herring collected in SEAK were the most temporally stable of any group sampled. Predictive DFAs were constructed for herring collected in SEAK in 2005 and 2006 (Table 16). Both models identified herring collected in 2001 as reliably as those collected in the adjacent year. On average, 90% of the samples could be correctly identified. The reliability of these models was further demonstrated by the nMDS with ANOSIM (Figure 5). Sitka Sound sample sites always mapped onto each other in the upper left hand portion of the figure. Hoonah Sound sample sites always mapped to the lower right hand portion. Moreover, the temporal component observed in other regional comparisons (e.g., NGA, Bering Sea) was not as strong in the comparisons between Sitka area sample sites. For example, the fatty acid compositions of herring collected from Sitka Sound in 2001 were more similar to those of herring collected there in 2005 than the 2005 Sitka Sound herring were to those collected from Hoonah Sound in 2005. This is a distinctly different result than was observed in any of the other regions.

Reliability of identifying stock of origin from winter samples

We performed DFA's on herring collected from different regions and on areas within regions and on individual sample sites within areas. We used these models to identify fish collected in the winter of 2007 from Kodiak Island (Table 1). The predicted regional affiliation for the 2007 NGA winter samples (aka Kodiak) was split between NGA, SEAK, and the Aleutian Islands, depending on which year of data was used to build the predictive DFA model (Table 12). ANOSIM R statistics revealed that winter 2007 Kodiak samples were significantly different from all other regions sampled (Table 13) and second stage nMDS plots corroborated that result, plotting the NGA-winter (aka Kodiak) sample far removed from all other areas sampled (Figure 2). In the area comparisons, winter 2007 samples from Kodiak were identified as Kamishak fish 93.3% of the time by the 2001 model, but only 30% and 86.7% of the time using the 2005 and 2006 models, respectively (Table 14). Second stage nMDS plots of herring collected in the NGA across years also showed considerable distance between Kodiak winter 2007 samples and all others collected, with the possible exception of the 2006 Kamishak Bay samples (Oil Bay and Nordyke Island; Figure 3).

Otolith Microchemistry

Regional Differences

We observed little evidence of stock structure across regions using otolith microchemistry. Discriminant functions were slightly better than random at identifying the correct sampling region for a known otolith left out of the dataset used to build the DF. Based on the composition of otolith cores, samples could be reliably identified to their region of origin 42% of the time. North Gulf of Alaska (NGA) samples were correctly identified 64% of the time, while only 17% of the SEAK samples were correctly identified (Table 17). Based on the composition of otolith edges, samples could be reliably identified 45% of the time, with NGA (74%) and SEAK (4%) once again representing the most and least successfully identified regions, respectively (Table 18). Moreover, there appeared to be little spatial structure to the erroneous identifications of either otolith cores or edges. Most misidentifications placed fish in either the NGA or Bering Sea regions (Tables 17 and 18).

The failure to identify stock structure on a regional basis was also evident in the nMDS plots. The plots indicated the core compositions were broadly spread along the first axis with no difference among regions (Figure 6). The second axis offered little resolution to this general pattern. R values from the ANOSIM further indicated little separation between regions. The greatest difference observed was between otoliths from the Bering Sea and Aleutians, but this value was 0.08 and not significantly different from 0 ($P > 0.05$; Table 19), indicating no detectable distance between the group centroids. In contrast, there was some evidence of regional stock structure when the edge compositions were considered (Figure 7). While the nMDS plot indicated a high degree of similarity among regions, R values were significantly greater than 0 ($P < 0.05$) in all three cases where the NGA was compared to other regions (Table 20). Despite the apparent isolation of NGA herring, samples from the Aleutian, SEAK, and Bering Sea regions were indistinguishable from one another ($R < 0.07$, $P > 0.05$).

Differences across areas and sites

The failure to detect spatially coherent differences on the regional level indicated little hope of seeing structure on finer scales. However, we examined differences in fish from Hoonah and Sitka Sounds, because these two sites were found to vary significantly in fatty acid composition. In addition, herring in this area have demonstrated significant variation in otolith chemistry under a separate Restoration Study (EVOS Project 070834; H. Woody, Sitka Tribe, personal communication). This comparison offered the best opportunity to identify stock differences in otolith composition on a fine spatial scale.

Otoliths from the Sitka and Hoonah Sound sites were discriminated with a somewhat higher degree of success than was had with regional comparisons. Both otolith core and edge chemistries provided similar results, with discriminant functions correctly identifying individuals 65-66% of the time (Tables 21 and 22). Generally, the models were better at identifying Hoonah Sound fish, particularly when edge compositions were considered (Tables 21 and 22). These results were consistent with those displayed by the nMDS plots (Figures 8 and 9). However, no difference in composition was detected by the ANOSIM for comparisons based on either core or edge chemistries ($P > 0.05$; $R < 0.08$).

Homing to natal areas

Comparison of the edges and cores of otoliths revealed significant changes in otolith composition between the core and edge that were consistent across sites. The second stage nMDS plot displays significant separation in otolith edges and cores taken as a whole (Figure 10), but no differences between sites. This clearly indicates that edge and core compositions do not match. ANOSIM supports the conclusion that otolith edges differed from cores ($P < 0.05$) in each of the ten within site comparisons. The R values for comparisons between core and edge ranged between 0.023 and 0.84 with a median value of 0.60. Moreover, cores tended to be more similar to each other than edges. Few differences were detected among sites when cores were compared (Table 23) and R values were uniformly low (<0.14). While the majority of comparisons between otolith edges also failed to detect differences among sites (Table 24), there was greater variation than was observed among cores. The edge chemistry of otoliths from two sites tended to be different from all others. The chemical composition of otolith edges from Iliulink Bay (Dutch Harbor) differed ($P < 0.05$) from six of the other nine sites examined. R values ranged from -0.01 to 0.23. Similarly, the chemical composition of otolith edges collected in Nunavachak Bay (Bristol Bay) differed ($P < 0.05$) from six of the nine other sites. In this case, R values ranged from -0.01 to 0.19 (Table 24).

We consistently found the Sr:Ca ratio of otolith cores to be significantly different from that of otolith edges across all locations sampled in 2006 (Figure 11). Life history transects run from the otolith core to the margin revealed that elevated concentrations of Sr and diminished concentrations of Mg occurred from the core to a distance of approximately 750 microns from the core. Sr and Mg levels were generally consistent from 750 microns, radial distance from the core, out to the otolith margin (Figure 12).

Ability to assign winter samples to spawning stocks

Stock identities of herring collected in Uganik Bay during the winter of 2007 depended on where otoliths were sampled (e.g., core vs. edge). We fit the data collected from samples collected on November 29, 2007 (i.e., Kodiak-Winter) to a DFA model derived from local (e.g., Kodiak Island and Kamishak Bay) spawning aggregations sampled in the Spring of 2006. Based on the composition of otolith cores, 26% of the Kodiak (Winter) herring were assigned to the Spring 2006 spawning sample from Uganik Bay (Table 25). Most of the remaining Kodiak (Winter) samples were classified as originating from either the Oil Bay or Nordyke Island spawning samples taken from Kamishak Bay in Spring 2006 (Table 25). When we used otolith edges to conduct a DFA to identify the stock of origin for Kodiak (Winter) samples, we got a somewhat different result. Forty-six percent of the herring collected in Uganik Bay during Winter 2007 were assigned to the Spring 2006 spawning sample from Uganik Bay (Table 26), and most of the remaining samples were assigned to Kiliuda Bay, on the East Side of Kodiak Island. Only 21% of the Kodiak (Winter) samples were assigned to Kamishak Bay spawning aggregates (Oil Bay; Table 26). These results were consistent with the nMDS plots depicting the spatial relationship between otolith core (Figure 13) and edge (Figure 14) chemistries.

DISCUSSION

Storage and handling of fatty acids and otoliths

To avoid lipid deterioration and oxidation of fatty acids when fish could not be sampled immediately after capture, we held them in totes of iced seawater. Anelich et al. (2001) found that no deterioration in lipid composition occurred in catfish fillets stored at 2º C for 13 days. Aidos et al. (2002) found very little change in percent free fatty acids of herring oil stored for 160 days at temperature ranging up to 50º C, however, the formation of primary oxidation products did occur gradually over time even when oil was stored at 0º C. In our study, hold time never exceeded 8 hours and generally less than 3 hours elapsed between a herring's harvest until it's heart tissue was placed in liquid nitrogen (-70º C). We monitored body core temperatures while samples were being processed and temperatures never exceeded 17º C and were generally kept between 2-8º C.

Brophy et al. (2003) found that otoliths from herring larvae stored frozen for a prolonged time had higher Mg and Zn levels than those frozen for less time. Milton and Chenery (1998) also reported higher Mg levels at the core and edge of Toli shad, *Tenualosa tola,* otoliths that had been stored frozen. Swan et al. (2006) found lower concentrations of Ba and Cr in otoliths from three marine species that had been stored frozen. Ruttenberg et al. (2005) was concerned about the impact different storage methods (e.g., frozen vs. ethanol) had on their study showing elevated levels of trace elements in the cores of otoliths from six different marine species. However, because their storage method was consistent within species and they were evaluating intraspecific patterns, they assumed storage method would not impact their overall conclusions. Similarly, all of the samples collected for this study were stored in a similar manner for similar times.

Use of Fatty Acids and Otolith Microchemistry for Stock Identification

Whether or not detection of stock-specific discernable differences in phenotypic traits (e.g., fatty acid composition and otolith chemistry) is evidence of population structure is open to debate (Waples 1998, Begg et al. 1999a, Begg and Waldman 1999). Given the ongoing debate over the type of questions fatty acid analysis can be appropriately used to answer (e.g., Thiemann et al. 2004, Grahl-Nielsen et al. 2004), some discussion of our rationale for investigating it as a stock identification tool is warranted.

It is clear that many studies have documented how fatty acid compositions can change with diet (e.g., Fraser et al. 1989, Kirsch et al. 1998, Turner and Rooker 2005, Budge et al. 2006). However, the ability to trace the dietary influence of some individual fatty acids is much greater than others. Turner and Rooker (2005) documented a 35% change in the poly unsaturated fatty acids (PUFA) of juvenile red drum (*Sciaenops ocellatus*) after just 5 days of controlled feeding. Fraser et al. (1989) observed the incorporation of dietary fatty acids into the triacylglycerols (TAG) of Pacific herring larvae in a marine enclosure over the course of 43 day feeding trial. Fraser et al. (1989) found that peak 18:4n-3 levels in phytoplankton transferred through zooplankton in an enclosed marine food chain and into herring larvae in about 23 days. Haugen et al. (2006) evaluated seasonal variations in muscle growth and fatty acid composition of Atlantic halibut (*Hippoglossus hippoglossus* L.) and found that the TAG fraction of the fatty acid

profile was most affected by diet while the polar fraction was less influenced. Clearly, diet is a major factor affecting the composition of fatty acids in fish.

Much of this work has focused on whole lipid or TAG; many studies that examine phospholipids have yielded different results. Fish stocks and strains have been differentiated using fatty acid analysis, even when they have been reared under identical conditions and fed identical diets (e.g., Joensen et al. 2000, Peng et al. 2003). Peng et al. (2003) reported that while great similarities were found in the fatty acid profiles of whole body TAG of two strains of Atlantic salmon fry, they observed marked genotypic differences in the PUFA profiles of whole body phospholipids. Pickova et al. (1997) investigated the lipid fatty acid composition of eggs from two cod stocks and concluded that the composition of phospholipids was more related to stock than to diet. Rottiers (1993) fed landlocked and anadromous strains of Atlantic salmon identical diets and found that landlocked strains had higher lipid content. Rollin et al. (2003) also studied diet effect on anadromous and landlocked Atlantic salmon parr and concluded that "differences in specific fatty acid concentrations between fish fed the same experimental diet may be due to their individual capacities for linolenic acid (LNA) conversion to longer and more saturated n-3 PUFA". They further suggested that differences in individual capacities to process fatty acids may have a genetic basis, but also noted that other researchers have found that temperature can influence the fatty acid composition of some phospholipids (e.g., Hazel 1984). Finally, in a cautionary note to other researchers, Rollin et al. (2003) reported that the significant differences they found in fatty acid composition between salmon strains was highly dependent on the specific fatty acids considered in the analyses.

We targeted heart tissues because heart phospholipids are reported to be less subject to environmental influences than other tissues or lipid classes (Grahl-Nielsen and Ulvund 1990, Czesny et al. 2000, McKenzie 2001). Several studies have shown that dietary impacts on fatty acid composition are minimized in heart lipids. Viga and Grahl-Nielsen (1990) cultured groups of Atlantic salmon from the same stock for eight months on prescribed diets and concluded that fatty acid composition of salmon hearts was independent of diet. This conclusion is not universally supported. Owen et al. (2004) reported that the fatty acid compositions of myocardial membranes in rats fed different diets were directly related to those of their food. McKenzie (2001) also reported the tendency for heart fatty acid composition to respond to diet, but at much lower magnitude than muscle or liver. These studies suggest that examination of heart fatty acids should minimize the apparent variation imposed on populations due to diet, ration, temperature, and salinity (Henderson and Tocher 1987, Grisdale-Helland et al. 2002, Kiessling et al. 2001, Cordier et al. 2002, Jobling et al. 2002).

The concept of genetic control over the composition of heart fatty acids is bolstered by studies demonstrating relationships between cardiac function and fatty acid composition. Bell et al. (1993) reported heart lesions in Atlantic salmon fed diets with high levels of n-6 fatty acids after the fish had been stressed. Agnisola et al. (1996) reported reduced heart rate and cardiac power output in the hearts of sturgeon fed diets high in n-3 fatty acids relative to those fed diets high in n-6 fatty acids. McKenzie (2001) reviewed evidence that dietary fatty acid composition can have a significant influence on the respiratory and cardiovascular physiology of farmed sturgeons and eels. McKenzie et al. (1999) reviewed a number of studies demonstrating that experimental diets fed to Adriatic sturgeon, *Acipenser naccarii*, influenced their cardiovascular physiology and

ability to tolerate hypoxic stress. Chatelier et al. (2006) reported that seabass, *Dicentrarchus labrax*, fed experimental diets that enriched their tissues with oleic and linoleic acids (OA, 18:1n-9 and LA, 18:2n-6, respectively) led to improved cardiorespiratory performance. These studies demonstrate an influence of heart fatty acid composition on individual fitness, thereby providing a basis for differences among reproductively isolated aggregates. Alternatively, interactions between phospholipid composition, eicosanoid production and cardiac function have rarely been described for fish (Stenslokken et al. 2002) despite their frequently described impacts on mammalian health (Das 2001). These data may account for the conclusion that some individual fatty acids (e.g., C22:6n3) in fish heart phospholipids are not strongly influenced by diet (Thomassen and Røsjø 1989, Caballero et al. 2002, Grisdale-Helland 2002), and in fact may be under strong genetic control (Peng et al. 2003), suggesting fatty acid analysis of heart tissue may be appropriate for investigating stock structure.

Similar to fatty acid analysis, some concern exists over the concept that stock-specific discernable differences in otolith chemistry are evidence of population structure. Campana (1999) cautioned that otolith chemistry differences don't infer genetic differences because larval dispersal can occur across population sub units. However, differences in otolith chemistry can indicate that fish have spent significant parts of their lives in different environments (Campana 1999), which may indicate diverse life history strategies that management policies would be prudent to maintain (Bergenius et al. 2005, Jonsdottir et al. 2006).

Spatial, Temporal and Biological Variability

Fatty acid analysis

Our results suggest that fatty acid analysis of heart lipids was a reliable method for discriminating putative herring stocks at multiple spatial scales (region, area, site) corresponding to linear separations of > 750 km (region), 250-750 km (area), and sometimes even 75-250 km (sample sites), as long as samples were compared within and not across years. In most cases, our *a priori* stock identities appeared to best describe the fatty acid data structure. DFA correctly identified the region of origin 99%, 86%, and 90% of the time in 2001, 2005, and 2006, respectively. ANOSIM R statistics revealed significant differences among all regions compared, except SEAK vs. NGA in 2001. ANOSIM R statistics also revealed significant differences in fatty acid composition at the area scale, except for Kamishak vs. PWS in 2005. Cross-validation success at the area scale varied among the areas sampled, ranging from 70-89%. When individual sample sites were compared within management areas, significant differences were sometimes found between samples collected just 75-250 km apart (e.g., Sitka Sound vs. Hoonah Sound; east [Kiliuda Bay] vs. west Kodiak [Uganik Bay]; and southern [Chenik/Nordyke] vs. northern Kamishak [Iniskin/Oil Bay]).

The results of within-year comparisons from this study were comparable to our pilot study (Otis and Heintz 2003). Our lower overall cross-validation success in this study is likely due to higher intra-population variability in fatty acid compositions observed as a result of sampling all members of the population. In our pilot study, we controlled samples for age, sex, and maturity, thereby reducing inherent differences in fatty acid composition that may derive from age related diet changes and gonad maturity (Henderson and Tocher 1987, Huynh et al. 2007).

We did not observe a high degree of temporal stability in fatty acid composition for most of the stocks sampled. Cross-validation of discriminant functions and nMDS with ANOSIM revealed considerable shifts in fatty acid composition across both short (1 year) and long (4-5 year) time periods. This lack of temporal stability in fatty acid composition was observed at all spatial scales, with some exceptions. At the regional level, discriminant functions derived from collections in one year could correctly identify fish collected from the Bering Sea in an adjacent year more than 80% of the time. These same models were less able to discriminate fish from the northern Gulf of Alaska and southeastern Alaska. However, misidentifications of these fish were rarely the result of assigning them to the Bering Sea, suggesting fish in these regions are more closely related to each other than either is to the Bering Sea. This same conclusion was drawn by Grant and Utter (1984) and O'Connell et al. (1998) using genetic analyses. At the area/site level, only Sitka and Hoonah sounds exhibited a high degree of temporal stability. The relatively high temporal stability we observed in Southeast Alaska is in stark contrast to that observed in the northern Gulf of Alaska and Bering Sea.

The temporal shifts in fatty acid composition we observed in most herring stocks could be caused by a number of factors. Henderson and Tocher (1987) reviewed a variety of dietary and environmental factors that affect the fatty acid composition of different lipid classes. Cordier et al. (2002) reported that salinity can play a significant role in modulating the activities of enzymes acting on lipid metabolism during their natural circannual cycles. Farmed sea bass (*Dicentrarchus labrax*) fed all year on the same industrial diet showed a significant correlation between water salinity and the percentage of 22:6n-3 observed in muscle phospholipids (Cordier et al. 2002). Disease states have also been shown to impact the cardiac muscle lipids and the physiology of lipid metabolism in humans, so it's quite possible that changes in chronic (e.g., disease) and acute (e.g., predators, lack of food) stress levels could also introduce temporal shifts in fatty acid composition within and among fish stocks (Pers. Comm., S. Trumble, Baylor University).

Our study is not the first to report temporal shifts in fatty acid composition among stocks sampled *in situ*. Kwetegyeka et al. (2006) documented temporal shifts in the fatty acid composition of Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) hearts sampled in Lake Victoria in September 2002 and June 2003. Walton and Pomeroy (2003) used blubber fatty acid profiles to detect inter-annual variations in the diets of two breeding colonies of gray seals (*Halichoerus grypus*). Despite the obvious difference in study organisms, Walton and Pomeroy's (2003) work has many parallels to this study. They too had previously demonstrated the ability to discriminate their target populations based on fatty acid profiles (Walton et al. 2000). Once that was established, they collected additional samples in subsequent years to investigate the temporal stability of each population's fatty acid profile. They found that one was highly variable while the other was temporally stable across three breeding seasons. In another distinct similarity to our own study, they also discovered trends in the distance and directionality of the fatty acid profile shifts they observed over time, as revealed by principal components analysis (PCA) plots. They hypothesized that such a result may occur if members of the population changed their diet in a similar manner.

The existence of diet effects on the fatty acid composition of heart phospholipids does not rule out genetic influences. Maintenance of myocardial membrane fatty acid compositions is essential for cardiac function and mitochondrial respiration (Hatch 2004). Three laboratory studies have reported evidence of a genetic component to fish fatty acid compositions. Joensen et al. (2000) found significant differences in the fatty acid profiles of heart tissue extracted from representatives of two cod stocks that had been reared for 19 months under identical diets and environments. Peng et al. (2003) compared the fatty acid compositions of anadromous and landlocked Atlantic salmon (*Salmo salar*) fry, fed identical diets throughout a 44-day feeding trial, and reported significant differences in their phospholipids. In a companion study, Rollin et al. (2003) concluded that differences in the fatty acid composition of different strains of Atlantic salmon resulted from variation in the rates of desaturation and elongation of linolenic and linoleic acids. This suggests that differences in the activities of enzymes that regulate phospholipid composition might explain the stock differences identified in our pilot study on herring (Otis and Heintz 2003), as well as other species examined in field studies (Grahl-Nielsen and Ulvund 1990, Grahl-Nielsen and Mjaavatten 1992).

It is important to recognize that environments and diets were tightly controlled in each of the aforementioned laboratory studies that suggested genetic control of fatty acid composition. The variety of mechanisms by which vertebrates can control the molecular composition of their membranes (Hatch 2004) indicates that fatty acid composition is a quantitative trait subject to polygenic control. Consequently, it is reasonable to expect an interaction between genetic and environmental influences (Stearns 1992). Holding environment constant allows for identifying genetic differences. Conversely, sampling a population in different environments allows for identification of environmental effects on fatty acid composition (e.g. Walton and Pomeroy 2003). In our study, neither environment nor genotype was held constant. Therefore, temporal shifts in the foraging environment of adult herring and mixing of genotypes are confounded. Both could have interfered with our ability to discriminate among spawning aggregates across years.

Otolith Microchemistry

We found little evidence of stock structure across regions when comparing the elemental composition of otoliths that were analyzed using LA-ICPMS. Very little separation was apparent among regions when we examined otolith cores; however, the NGA region appeared to be significantly different from all others sampled when we compared otolith edges. This statistically significant result was not corroborated by ANOSIM R values and nMDS plots, which indicated very little difference among regions, regardless of the zone of the otolith analyzed.

These results contrast somewhat with those of our pilot study (Otis and Heintz 2003), in which we used an electron microprobe (EM) to identify significant differences in the elemental composition of herring otoliths from Sitka, NGA, and Togiak (MANOVA, $P < 0.0001$), and even among some areas within the NGA region (e.g., Kodiak and PWS-Montague grouped together, as did Kamishak and PWS-NE; Otis and Heintz 2003). The regional and area scale differences observed in our pilot study appeared to be a function of the relative percentages of Cl and Na (ANOVA, $P < 0.004$; Otis and Heintz 2003). Severin et al. (1995) also found Na (and K) to

provide the strongest discriminatory power in their EM analysis of juvenile walleye Pollock, *Theragra chalcogramma,* otoliths sampled from locations in the GOA and Bering Sea.

The contrast in results between our pilot study and this study may be due to several factors, including: differences in the instruments used, elements analyzed, or temporal effects. The EM and LA-ICPMS are two very different instruments. The detection limits for the EM are approximately 100 microgram/gram, while LA-ICPMS detection limits are generally 0.1-1 microgram/gram (Fitzgerald et al. 2003). EM analysis is therefore limited to measuring a few minor elements (e.g., Na, Cl, K, and Sr; Campana et al. 1997), many which are thought to be controlled by physiological rather than physicochemical factors (Campana 1999). The ICPMS is generally able to measure trace elements (Mg, Mn, Ba), most of which are thought to be more closely tied to environmental factors (e.g., water chemistry, temperature; Campana 1999) and may therefore be more appropriate for identifying groups of fish occupying different water masses.

Another potential reason why we found little evidence of structure across regions using LA-ICPMS may have to do with the elements we were able to successfully quantify. We detected but could not reliably resolve concentrations of Mn and Ba from background for all the sites we sampled. The analyses we performed require that there be no missing data in the vectors we are comparing. Hence, we excluded Mn and Ba from our analyses. Several authors have reported that successfully discriminating among sample groups across different spatial scales can largely depend upon the elements analyzed (e.g., Patterson et al. 2004, Bergenius et al. 2005). In a study involving coral reef fish, Bergenius et al. (2005) found that Ba provided good separation at fine spatial scales ($\leq$ 10's of km), while Sr and Mn were the elements most influential for defining regional scale differences (> 100's of km). Bergenius et al.'s (2005) results contrast with those of Patterson et al. (2004), who examined the post settlement part of the otolith to classify newly settled damselfish, *Pomacentrus coelestis,* on the Great Barrier Reef. Patterson et al. (2004) reported that Ba was best for defining structure between regions, Mn for discriminating reefs within regions, and Sr for sites within reefs.

As fish age, their physiological processes and behaviors often change creating the potential for alteration in the manner in which chemicals are incorporated into the otolith matrix. In our pilot study we controlled for age, sex, and gonad maturity and in the present study we randomly sampled among all members of the population. By controlling for age in our pilot study, we effectively removed any within and across group temporal effects that may have confounded our present analyses. Temporal effects may also help to explain why our edge data had greater discriminatory power than our core data. By targeting the edge, which represents a uniform capture time (Spring 2006), we hoped to remove the strong temporal component that existed for otolith cores sampled from fish ranging in age from 2-15 years (see Hamer and Jenkins 2007). However, we recognize the fact that fish collected at a spawning site must reside there for some period of time before the otolith edge can reflect the signature of the water chemistry. Munro et al. (2008) found that it took one to three weeks for the otolith chemistry of Golden perch, *Macquaria ambiqua,* fingerlings to reach equilibrium with their environment. Several authors have reported differences in the otolith chemistry of fish sampled at the same locations across various time spans (Swearer et al. 2003, Bergenius et al. 2005). Swearer et al. (2003) found temporal and species specific differences in otolith chemistry among five species sampled across

two seasons in three temperate estuaries in southern California. Bergenius et al. (2005) used solution based ICMPS to measure the chemical composition of entire otoliths and suggested that the variation in chemical signatures among older fish of different ages would likely be greater than that of like-aged juvenile fish from the same stock. Temporal variation in water chemistry is the most likely source for differences in otolith chemistry in fish sampled from the same locations over time. Elsdon and Gillanders (2006) found considerable variation in elemental concentrations of water in a tidal estuary between seasons, weeks, and days, for a majority of elements.

Concern over variation in otolith chemistry resulting from temporal, ontogenetic, and physiological differences has led some authors to recommend comparing signatures among individuals of the same age when using otolith chemistry to investigate stock structure (Hamer and Jenkins 2007). Hamer and Jenkins (2007) also recommended sampling the most recently deposited otolith material along the outer margin of same age fish to control for heterogeneous chemical signatures that might be caused by fish occupying changing physical environments. Given our results, we concur with Hamer and Jenkins (2007) and recommend a cohort analysis based approach be employed for future otolith studies focused on herring stock identification in Alaska.

Identifying stock of origin for winter food/bait samples

One of our objectives was to attempt to identify the stock of origin for fish sampled outside of their spawning period, such as during food/bait fisheries traditionally conducted during fall and winter. Because these fisheries occur during a time of year when herring are often aggregated in deepwater overwintering areas, there is a greater potential for these fisheries to target mixed stocks. Letcher and King (1999) suggested that the success and applicability of mixed stock analysis to a particular problem depends on four criteria. Although these criteria were stated in the context of the genetic analyses they were conducting, they can be generally applied to any mixed stock analysis:

1) level of differentiation among stocks,
2) ability to sample all (or virtually all) of the potentially contributing stocks,
3) the temporal stability of the markers chosen,
4) a large mixture sample size that contains representatives from most donor stocks.

These factors are not mutually exclusive, and it is possible that all four contributed to our difficulty in determining the stock of origin for Kodiak winter samples using fatty acid analysis; however the lack of temporal stability that we observed in this study was probably the primary contributing factor. We attempted to target the same spawning aggregations each sample year to minimize variability introduced by sampling different spawning waves. Ware and Tanasichuk (1989) reported that herring tend to spawn in discrete waves, with the largest fish spawning first and smaller fish later. Otis et al. (1998) reported a significant shift in the age composition between early and late spawning herring in Kamishak Bay. Older, repeat spawning cohorts dominated the early season and younger, recruit-aged cohorts dominated the late season. Hence, temporal differences introduced by sampling different cohorts and spawning waves across sample years may have contributed to the relatively high variability we observed in fatty acid signatures within sample groups.

Another source of "noise" potentially contributing to our difficulty in identifying winter samples may be related to spawning condition. Samples used to construct DFA models to identify the source of winter caught fish were necessarily collected during spring spawning to assure knowledge of stock of origin. However, Henderson et al. (1984) documented dramatic decreases (76%) in lipid content of muscle in male and female capelin during four months of gonadal development leading up to spawning. They also found a progressive increase in the percentage of long-chain monoenes 20:1 and 22:1 in the muscle lipids of both sexes. Huynh et al. (2007) recently reported that spawning and non-spawning herring also exhibited considerable differences in the relative distribution of individual fatty acids. These findings highlight the difficulty of matching baseline samples collected from spawning adults to samples collected at other times of the year. However, we attempted to minimize the effect diet and condition may have on fatty acid composition by focusing our analyses on phospholipid rich heart tissue.

As with the FAA, it's possible that all four of Letcher and King's (1999) above-referenced criteria contributed to our difficulty using otolith elemental analysis to determine the stock of origin for the Kodiak winter samples. However, the lack of significant differentiation among sample groups was certainly the primary contributor. The elements we were able to quantify using LA-ICPMS simply did not provide sufficient discriminatory power among our sample groups. Temporal stability issues may have also contributed to the variability we observed within sample groups being equal to or greater than the variability we observed among sample groups, because we did not control for age in our study. Recently, authors have recommended that otolith studies should target like-aged fish when the results are to be used for stock identification (Hamer and Jenkins 2007).

Based on our relatively poor success in correctly identifying known-origin spawning samples across years, it would appear that temporal stability issues confounded our ability to identify putative mixed-stock samples collected outside of the spawning season. However, it's important to note that because we don't know the true stock composition of the Kodiak (Winter) sample, we really don't know whether our analyses failed. It is interesting to note that comparison of the fatty acid discriminations using the 2006 model (Table 14) provided similar results to the otolith core models (Table 25). In both cases, Kodiak (Winter) samples were identified as deriving from Kamishak Bay in the majority of cases (87% and 69%, respectively.)

Homing to natal areas

Many studies have used the otolith core to represent natal rearing sites (e.g., Thorrold et al. 2001, Chittaro et al. 2006); however, we chose to analyze a target area just outside of the core due to concerns regarding potential maternal effects (e.g., Volk et al. 2000). MacDonald et al. (2008) define the otolith core as "the region bounded by the first prominent growth zone, encompassing the initial deposition site of the otolith calcium carbonate matrix known as the primordium". Brophy et al. (2004) reported that the primordium develops in fish during the embryonic stage, soon after fertilization, and is composed of protein and calcium rich granules that form the kernel around which subsequent calcification of the otolith occurs. MacDonald et al. (2008) used LA-ICPMS on age 0 and 1 Australian smelt, *Retropinna semoni*, to examine natal chemistry and recommended identifying the onset of daily growth rings to sample a target region that excludes

the primordium. We targeted an area 100-200 microns from the primordia where we consistently observed the first growth rings, which we assumed represented the onset of feeding after the yolk sac had been absorbed and while the larvae was still rearing in its natal spawning area.

Several recent studies have reported elevated levels of some elements, most commonly Mn, in the core region of otoliths from marine fish (e.g., Ruttenberg et al. 2005, Ludsin et al. 2006, MacDonald et al. 2008), including clupeids (Brophy et al 2004). Ruttenberg et al. (2005) found Mg and Ba to also be slightly elevated within the cores of six different species of marine fish. MacDonald et al. (2008) found slightly elevated Mg ratios and slightly depressed Ba ratios within the primordia region of Australian smelt *Retropinna semoni*, whereas Sr and Ca remained relatively constant. Influence from the mother has been offered as one potential explanation for elevated levels of elements in the otolith core (Volk et al. 2000, Brophy et al 2004), especially within the primordia (MacDonald et al. 2007). Brown and Severin (2009) found a Sr:Ca spike in the core region of an anadromous three-spined stickleback, *Gasterosteus aculeatus*, which they attributed to an anadromous mother. Thorrold et al. (2006) artificially injected a Ba isotope into female benthic and pelagic spawning marine fish and demonstrated maternal transmission to the otolith cores of their young. Several authors have therefore recently suggested that enrichment of the otolith core due to influences other than the environment (e.g. yolk sac input, changes in otolith crystal structure) may bias the use of otolith core chemistry to identify natal rearing sites (e.g., Ruttenberg et al. 2005, Chittaro et al. 2006, MacDonald et al. 2008). These studies corroborate our decision to target the area just outside of the core to represent natal rearing sites.

We attempted to determine whether adult herring homed back to their natal rearing area to spawn by comparing the chemical composition of the otolith edge (representing spawning sites) to the core (representing natal rearing sites) on otoliths we collected from spawning fish in 2006. We consistently found the chemical composition of otolith cores to be significantly different than that of otolith edges across all locations sampled, which confounded our ability to draw any conclusions about natal homing. So called "life history transects" that run from the otolith core to the margin revealed an apparent ontogenetic effect on Sr and Mg. We observed elevated concentrations of Sr and diminished concentrations of Mg to occur from the core to a distance of approximately 750 microns from the core. Sr and Mg levels were generally consistent from 750 microns out to the otolith margin. Chitarro et al. (2006) also reported that the concentrations of several elements (e.g., Mn, Zn, Sn, Ba, Ce, and Pb) were significantly greater in embryo otoliths (i.e., essentially the otolith core) than in the edge signatures of juvenile damselfish *Stegastes partitius*, collected at the same site and time. As a result, they cautioned using otolith core signatures as a proxy for natal signatures.

Several authors have reported differential uptake of elements into otoliths of different species occupying the same environments (e.g., Swearer et al. 2003, Hamer and Jenkins 2007) and others have suggested ontogenetic factors may influence the incorporation of elements within species (Brophy et al. 2004, Chittaro et al. 2006, Brown and Severin 2009). Campana (1999) reviewed many studies that indicate the physical environment fish occupy is not the only factor influencing the composition of trace elements in their otoliths. Brown and Severin (2009) reported positive ontogenetic trends in Sr:Ca ratios for most of the marine species they

examined, with the maximum difference between otolith core and margin sometimes exceeding 6 mmol:mol, similar to the extreme range observed for some diadromous species.

The fact that we observed ontogenetic shifts in the ratios of Sr and Mg to Ca is not unusual. However, the direction of the trends we observed is contrary to those reported for many other species (e.g., Sadovy and Severin 1994, Campana 1999, Hamer and Jenkins 2007, Brown and Severin 2009), and is also counter to the general understanding of how uptake of these two elements is thought to be influenced by otolith calcification rates (Campana 1999, Hamer and Jenkins 2007). Higher calcification rates (i.e., periods of faster growth) are generally expected to result in lower Sr:Ca ratios because Sr is thought to substitute directly for Ca in the aragonite lattice (Campana 1999, Sinclair 2005). In contrast, Mg is expected to increase with otolith accretion rates because Mg likely gets trapped in lattice defects in the aragonite crystal and these defects increase with calcification rates (Sinclair 2005, Hamer and Jenkins 2007). Hence, one would expect to see depressed Sr and elevated Mg levels near the core of the otolith, where growth is fastest. This generalized trend is not common to all species. Brown and Severin (2009) examined the Sr:Ca profiles of dozens of freshwater, diadromous, and marine species to evaluate the extent to which water chemistry influences otolith chemistry. Although most species they examined exhibited positive Sr:Ca trends from otolith core to edge, Pacific herring collected from the Gulf of Alaska exhibited a trend that was consistent with our data (Pers. Comm., R. Brown, USFWS). Campana (1999) reviewed the array of interacting factors (e.g., salinity, temperature, growth rates) that appear to influence incorporation of Sr into otoliths and concluded that further research is clearly needed before a detailed hypothesis can be developed to explain otolith Sr:Ca ratios.

Herring population structure

While it is universally accepted that knowledge of population structure is relevant to successful management of exploited populations, there is no such agreement over what defines a "population". Waples and Gaggiotti (2006) discuss the continuum of interconnectedness of sub-populations from discrete (isolated) to fully mixed (panmixic) and emphasize that researchers need to identify their stock identification efforts with one of two population paradigms-evolutionary or ecological. In the ecological paradigm, the emphasis is on spatio-temporal co-occurrence so individuals can interact demographically (e.g., competition, social, and behavior interactions), whereas in the evolutionary paradigm the cohesive forces are primarily genetic and emphasis is on reproductive interactions between individuals (Waples and Gaggiotti 2006).

Our study falls under the ecological paradigm, which Waples and Gaggiotti (2006) describe as being more challenging to analyze, but also more relevant to questions relating to demographics and management boundaries. Our goal was to evaluate tools that may allow us to identify ecologically significant differences among adjacent spawning aggregations so fishery managers can refine the methods with which they assess and manage exploited herring stocks. Due to the ongoing difficulty of clearly defining herring stock structure, the current assignment of fishery management boundaries in Alaska may reflect political/administrative considerations as much as it does ecologically significant population structuring. It was with the hope of improving that situation that we undertook this study.

Considerable research has been focused on determining the spatial scale at which spawning fidelity and stock structure exists in herring (e.g., Hay and McCarter 1997, Ware et al. 2000). In the absence of more definitive tools, many fishery managers have traditionally used spawning timing and location as proxies to roughly define herring stock structure. The logical assumption is, the greater the temporal and spatial separation between spawning aggregates, the greater the likelihood that they are discrete stocks. Some researchers have reported that there is evidence to suggest that spawn timing is not heritable and is very plastic (Smith and Jamison 1986). However, recent work has demonstrated that the clearest genetic distinctions found among closely related population sub-units were for those groups whose spawning period was the most temporally isolated from adjacent population sub-units, or whose spawning area was the most physically isolated (e.g., Small et al. 2005). McPherson et al. (2004) found that the temporal proximity of collection dates explained 30% of the pair-wise population differentiation ($P=0.0025$) they observed in Atlantic herring. Brophy et al. (2006) reported that autumn and winter-spawned Irish and Celtic sea Atlantic herring maintained their spawning season fidelity despite extensive mixing of the strains during their juvenile phase, suggesting that spawning timing for some populations is a genetic rather than a learned response.

Using fatty acid analysis, we were able to discriminate putative herring stocks at both broad (>750 km) and fine (<250 km) spatial scales. Not surprisingly, the highest values we observed for cross-validation success of DFA models were at the regional scale (>750 km). We found clear differences among within-year fatty acid samples collected from Southeast Alaska, the North Gulf of Alaska, and the Bering Sea. Grant and Utter (1984) and O'Connell et al. (1998) also documented genetic divergence between Gulf of Alaska herring and Bering Sea herring, however, O'Connell et al. (1998) was unable to find similar divergence among putative stocks of NGA herring. While we were able to successfully discriminate NGA stocks with reasonably good success within years, we did see evidence of interconnectedness among population subunits at this scale. And although we also found clear differences between some sample sites separated by less than 100 km, temporal spacing of sample collection may have played a bigger role than spatial distance between sites in distinguishing samples.

Other researchers have identified spatial structure in herring populations at similarly fine scales (e.g., Small et al. 2005); however, most often evidence of stock structure is only apparent at larger scales. Hay et al. (2001) used over 40 years of tag return data to investigate homing, geographic fidelity, and straying of Pacific herring along the British Columbia coast. The authors distinguished between natal homing, where fish home back to where they were born, and "sexual" or "reproductive homing" where fish home back to the same spawning area annually. Hay et al. (2001) reported fidelity rates were as high as 81.7% at the regional scale (10,000 km^2), but only 57.5% at the scale of their statistical reporting areas (500-2,500 km^2). They found little evidence of fidelity at scales less than 500 km^2 and suggested geographic scales of 1,000 linear km of coastline were needed to see fidelity rates in the 60-80% range. Similar scales of fidelity were observed in Atlantic herring by Wheeler and Winters (1984).

Hay et al.'s (2001) distinction between natal homing and reproductive homing is particularly relevant to herring. McQuinn's (1997) "adopted migrant hypothesis" suggests that recruit spawning herring don't return to their natal spawning area due to genetic imprinting, but instead follow older repeat spawning fish in the school back to a spawning location which they then

home back to in subsequent years. This hypothesis is supported by observations of Norwegian spring-spawning herring where changes in migration pattern generally coincide with the recruitment of abundant year classes to the spawning population.

One final consideration regarding the interconnectedness of populations and the scale at which population structure exists involves the issue of larval advection. Gaughan et al. (2001) reported potential links between distant management units (> 1,000 km) for larval *Sardinops sagax* that could be passively transported by prevailing ocean currents in southern and western Australia. Along with straying of adults (Hay et al. 2001), larval advection may account for some of the difficulty traditional genetic tools have had in distinguishing putative herring stocks at relatively fine spatial scales (<1,000 km; e.g., O'Connell et al. 1998). It may also explain some of the similarities we observed in the fatty acid compositions of putative stocks sampled across regions and management areas (e.g., NGA and Aleutian Islands, Kamishak and Kodiak, Togiak and Kuskokwim Bay). In the NGA, the prevailing current runs up the East side and down the West side of Cook Inlet and then follows the coastline down Shelikof Strait and out along the south side of the Alaska Peninsula (Burbank 1977). This introduces the possibility that Kamishak origin herring larvae may be advected to the Kodiak Management Area, and Kodiak herring may be advected to the Alaska Peninsula-Aleutian Islands Management Area, providing a possible explanation for the fatty acid profile similarities we found between Kamishak /Kodiak and Kamishak/Kodiak/Dutch Harbor.

Management Implications

Comparisons between the fatty acid compositions of fish collected from the Sitka Sound and Hoonah Sound sites in Southeast Alaska indicated the sort of temporal stability that could be useful for future life history studies of these stocks (e.g., determination of home range, over-wintering areas, and larval dispersal patterns). It may also be useful to fishery managers interested in further defining stock structure issues in Southeast Alaska, as well as determining the stock composition of mixed stock samples collected away from natal spawning areas.

The commercial sac-roe fisheries that target herring spawning in Kamishak Bay (Cook Inlet) and the northwestern side of Kodiak Island (Shelikof Strait) are managed separately, however, there is evidence suggesting these stocks may inter-mix in northern Shelikof Strait as rearing juveniles and over-wintering adults (C. Burkey, Alaska Department of Fish and Game, unpublished scale pattern analysis data). Consequently, the Kamishak Bay District Herring Management Plan [Alaska Administrative Code (AAC) 5 AAC 27.465] allocates a portion of Kamishak Bay's harvestable surplus to the Kodiak area food/bait fishery under the assumption that Kamishak origin fish may be harvested during fall/winter in some of the northern Shelikof districts targeted by the Kodiak food/bait fishery.

The temporal shifts in fatty acid composition that we observed in most populations sampled made it difficult to identify stock of origin for the putative mixed-stock sample we collected from Kodiak Island (Uganik Bay) during Winter 2007. Second stage nMDS plots and ANOSIM R statistics comparing herring collected throughout the NGA indicated that the Kodiak Winter 2007 sample was significantly different from all NGA samples collected in 2005 and 2006. However, in both our pilot study (Otis and Heintz 2003) and the current study, Kamishak and Kodiak were the areas most often confused with one another during within-year comparisons of

NGA samples. nMDS with ANOSIM results revealed that Kodiak and Kamishak herring shared the most similar fatty acid compositions among the areas compared. This result may simply derive from the close spatial juxtaposition of these two stocks and the similar time frame that samples were collected. However, our results provide no evidence that is contrary to the current theory that Kodiak and Kamishak herring stocks intermix during a part of their life history and Kamishak origin herring may be vulnerable to harvest during the Kodiak winter food and bait fishery. Hence, we found no cause for recommending modification of the Kamishak District Herring Management Plan at this time.

Similar issues surround herring management in Togiak, and Dutch Harbor. The Bristol Bay Herring Management Plan [5 AAC 27.865] allocates a portion of the Togiak District sac-roe herring harvestable surplus to the Dutch Harbor food and bait fishery under the assumption that harvests from each fishery derive from a single stock. In contrast, the results of this study indicated that the Dutch Harbor sample was easily distinguishable from all other regions (i.e., cross-validation success was 100%) and nMDS with ANOSIM revealed the Dutch Harbor sample to be more similar in composition to NGA and SEAK than Bering Sea. This finding contradicts previous assumptions regarding the affinity between Togiak (Bristol Bay) and Dutch Harbor herring stocks (Pers. Comm. T. Baker, ADF&G, Bristol Bay Area Research Biologist) and would appear to provide incentive to revise the Bristol Bay and Dutch Harbor herring fishery management plans. However, several key factors undermine the basis for drawing such a strong conclusion from our FAA results. Principal among them are the results of two scale pattern analysis studies that found 73%-88% of the herring sampled in Dutch Harbor belonged to Bering Sea spawning stocks (e.g., Togiak, Nelson Island, Port Moller; Walker and Schempf 1982; Rogers et al. 1984). ADF&G biologists in Kodiak and Dutch Harbor also indicate that the vast majority of herring arriving in the Dutch Harbor area in July are post spawning adults (Pers. Comm. M. Witteveen, ADF&G, Kodiak Area Research Biologist). Indeed, 100% of the herring that we sampled from Dutch Harbor for this study were “spent” and many had been actively feeding, which may have affected their fatty acid composition. This latter point, combined with the fact that the Dutch Harbor sample was collected 2-3 full months later than Togiak and NGA samples, seriously undermines our ability to draw conclusions from our FAA results. Given the weight of evidence to the contrary, we feel it’s inappropriate to conclude that Dutch Harbor and Togiak represent discrete stocks, based on our fatty acid data. However, we do recommend that further stock identification work be done to determine what portion of the Dutch Harbor area’s overall summertime biomass may derive from local, discrete spawning stock(s).

Previous efforts to identify population structure among PWS herring using microsatellite DNA variation found little genetic divergence among the four spawning aggregations sampled, with the possible exception of Port Chalmers (O’Connell et al. 1998). Our study also had difficulty finding evidence of fine scale structuring among spawning aggregations in PWS using fatty acid analysis and otolith microchemistry, however, our analyses were likely compromised by limited sample sizes and an inability to collect samples representing all historically important spawning aggregations. Currently, temporally and spatially separated spawning aggregations in PWS are managed as a single stock [5 AAC 27.365(b)]. Similar uni-stock management strategies are in place for other sac-roe herring fisheries around Alaska (e.g., Kamishak and Togiak). In an alternative approach, the total forecasted harvest for the Kodiak Management Area is apportioned among more than 75 individual sections within fourteen management districts [5

AAC 27.535]. This broad disparity in management approaches is characteristic of the lack of consensus found among herring researchers and managers regarding the spatial scale at which stock structure exists.

Hilborn et al. (2003) emphasized the important role "biocomplexity" has played in fisheries sustainability using the Bristol Bay sockeye salmon stock complex as a case study. Ruzzante et al. (2006) suggested that resistance to disturbance (natural or anthropogenic) may be compromised if genetic diversity (and by extension, biocomplexity) is reduced through management practices that disproportionately impact smaller, currently less productive sub-populations. Secor (2007) provided many examples of marine species that exhibit temporal and spatial spawning diversity and similarly argued that intra-population "biocomplexity" favors population resiliency. Regardless of the lack of a definitive tool for clearly defining herring population structure in Alaska, a strong case can be made for management strategies that maintain intra-population temporal and spatial spawning diversity.

CONCLUSIONS

We conclude that heart tissue fatty acid profiles can be used to discriminate Alaskan herring stocks at relatively fine spatial scales when models are built from data representing spawning fish collected within the same year, but they cannot be used to reliably discriminate unknown samples collected outside the spawning season (e.g., from mixed stock fisheries) or across years. The failure of fatty acid analysis to be effective as a mixed-stock analysis tool for most stocks is disappointing; however, its value for discriminating fine scale structure over short time periods should not be undervalued. In the near term, fatty acid analysis may be useful towards further resolving a number of pressing management questions regarding stock structure in southeastern Alaska, Prince William Sound, Kodiak/Kamishak, and perhaps Togiak/Dutch Harbor, as long as samples are collected in the same spawning season.

We also found that Southeast Alaska herring stocks exhibited much greater temporal stability in fatty acid composition than any other region sampled. If future studies corroborate that finding, and perhaps identify other stocks with similar temporal stability, then fatty acid analysis could be used as a basis for understanding the home ranges, over-wintering areas, and larval dispersal patterns for these stocks. We recommend further investigation of the intra- and inter-annual temporal stability of Southeast Alaska herring stocks. To clarify the relative contribution that genetic and environmental (e.g., diet) influences have towards determining heart tissue fatty acid composition, we also recommend that controlled laboratory feeding trials be conducted using experimental groups comprised of genetically isolated herring populations (e.g., Sitka, Togiak). Through such work it may be possible to identify individual fatty acids that are less influenced by diet, are under stronger genetic control, and may therefore be useful towards future herring stock identification studies.

Although we had poor success identifying population structure at various spatial scales using otolith microchemistry, we are reluctant to suggest that this technique shouldn't be used in future herring stock identification studies. This conclusion is derived mainly from the fact that the power of our analyses was limited by 1) the low number of elements we successfully quantified,

2) our difficulty in controlling for temporal effects, and 3) our uncertainty regarding the length of residency on the spawning grounds and how that affected whether the capture location (i.e., natal spawning area) was represented by the otolith edge chemistry. We recommend future otolith studies: 1) employ methods that assure a greater number of elements (i.e., response variables) are available for statistical analyses, 2) employ cohort analyses to help control for temporal effects, and 3) supplement otolith chemical analyses with an examination of otolith morphology (e.g., microstructure and shape analysis), which have been show to improve the effectiveness of using otoliths for stock identification.

Several herring management areas throughout Alaska contain temporally and spatially separated spawning aggregations that are currently considered to comprise a single stock (e.g., PWS, Kamishak, and Togiak). While considerable uncertainty continues to exist regarding the ecological significance of population "sub-units" (Stephenson 1999), there has recently been a growing recognition of the interconnectedness of marine fish populations (e.g., Hauser et al. 2005, Waples and Gaggiotti 2006, Benson et al. 2007) and of the inherent resilience that stock complexes gain when biocomplexity is maintained (e.g., Hilborn et al. 2003, Ruzzante et al. 2006, Mitchell 2006, Secor 2007). We recommend fishery managers consider revising herring management plans that don't explicitly recognize the value of temporally and spatially separated spawning aggregations by attempting to spread the harvest accordingly.

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Table 1. Region, area, site, and date(s) from which fatty acid, otolith, and genetic samples were *collected* for a Pacific herring stock identification study in Alaska, 2005-2007.

Sample Strata				Samples Collected					
				Fatty Acid		Otolith		Fin Clips	
Region	Area	Site	Sample ID	Qty[a]	Date(s)	Qty[a]	Date	Qty	Date
Southeast	Sitka	Sitka Sound	SITKA 1	139	3/20/05, 3/24/06	73	03/24/06	73	03/24/06
(SEAK)		Hoohah Sound	SITKA 2	115	4/25/05, 4/25/06	56	04/25/06	56	04/25/06
N. Gulf of Alaska	Prince William Sound	Gravina (Hells Hole)	PWS 1	129	4/7/05, 3/27/07	61	03/27/07	61	03/27/07
(NGA)	(PWS)	Gravina (St. Mathews Bay)	PWS 2	72	04/04/07	72	04/04/07	72	04/04/07
	Kamishak Bay	Chenik/Nordyke	KAMISHAK 1	139	5/2/05, 5/18/06	73	05/18/06	73	05/18/06
		Iniskin/Oil Bay	KAMISHAK 2	142	5/16/05, 5/21/06	73	05/21/06	73	05/21/06
	Kodiak Island	Uganik Bay	KODIAK 1	144	4/15/05, 4/17/06	75	04/17/06	75	04/17/06
		Uganik Bay (Winter)	KODIAK WINTER	69	12/31/05[b], 11/29/2007	69	11/29/07	69	11/29/07
		Kiliuda Bay	KODIAK 2	147	4/29/05, 5/5/06	75	05/05/06	75	05/05/06
Aleutian Islands	Dutch Harbor	Iliulink Bay	DUTCH 1	30	07/17/06	30	07/17/06	30	07/17/06
Bering Sea	Bristol Bay	Nunavachak	TOGIAK 1	136	5/4/05, 5/14/06	73	05/14/06	73	05/14/06
		Hagemeister Island	TOGIAK 2	132	5/4/05, 5/15/06	71	05/15/06	71	05/15/06
	Kuskokwim Bay	Goodnews Bay	BERING 1	75	5/8/05, 5/24/06[b]	0	05/24/06	0	05/24/06
		Tooksook/Nelson Is.	BERING 2	150	6/6/05, 6/9/06	72	06/09/06	72	06/09/06
			Total Sample Size:	1,619		873		873	

[a]Approximately 30 fish from each sampling event were randomly selected for analyses.

[b]Sample not viable.

Table 2. Region, area, site, and date(s) from which fatty acid, otolith, and genetic samples were *processed* for a Pacific herring stock identification study in Alaska, 2005-2007.

Sample Strata				Samples Processed					
				Fatty Acid		Otolith		Fin Clips	
Region	Area	Site	Sample ID	Qty[a]	Date(s)	Qty[a]	Date	Qty[c]	Date
Southeast (SEAK)	Sitka	Sitka Sound	SITKA 1	49	3/20/05, 3/24/06	25	03/24/06	0	03/24/06
		Hoohah Sound	SITKA 2	50	4/25/05, 4/25/06	21	04/25/06	0	04/25/06
N. Gulf of Alaska (NGA)	Prince William Sound (PWS)	Gravina (Hells Hole)	PWS 1	24	4/7/05, 3/27/07	11	03/27/07	0	03/27/07
		Gravina (St. Mathews Bay)	PWS 2	0	04/04/07	11	04/04/07	0	04/04/07
	Kamishak Bay	Chenik/Nordyke	KAMISHAK 1	57	5/2/05, 5/18/06	21	05/18/06	0	05/18/06
		Iniskin/Oil Bay	KAMISHAK 2	57	5/16/05, 5/21/06	25	05/21/06	0	05/21/06
	Kodiak Island	Uganik Bay	KODIAK 1	57	4/15/05, 4/17/06	21	04/17/06	0	04/17/06
		Uganik Bay (Winter)	KODIAK WINTER	29	12/31/05[b], 11/29/2007	25	11/29/07	0	11/29/07
		Kiliuda Bay	KODIAK 2	57	4/29/05, 5/5/06	27	05/05/06	0	05/05/06
Aleutian Islands	Dutch Harbor	Iliulink Bay	DUTCH 1	29	07/17/06	22	07/17/06	0	07/17/06
Bering Sea	Bristol Bay	Nunavachak	TOGIAK 1	49	5/4/05, 5/14/06	25	05/14/06	0	05/14/06
		Hagemeister Island	TOGIAK 2	50	5/4/05, 5/15/06	27	05/15/06	0	05/15/06
	Kuskokwim Bay	Goodnews Bay	BERING 1	27	5/8/05, 5/24/06[b]	0	05/24/06	0	05/24/06
		Tooksook/Nelson Is.	BERING 2	57	6/6/05, 6/9/06	22	06/09/06	0	06/09/06
			Total Sample Size:	592		283		0	

[a]Approximately 30 fish from each sampling event were randomly selected for analyses.

[b]Sample not viable.

[c]Fin clip samples were archived in ethanol for future genetic analysis.

Table 3. Mean lengths, weights, range of ages, and sample sizes for Pacific herring from which otolith chemistry and fatty acid composition was determined from fourteen collections sites in Alaska during 2005-2007.

Sample ID	Fork Length	Standard Length	Weight	Min. Age	Max. Age	n	Comments
Bering 1		279	315	4	12	27	
Bering 2		258	234	3	12	57	
Dutch 1		315	428	7	13	25	
Kamishak 1		222	123	3	7	57	
Kamishak 2		221	164	3	7	57	
Kodiak 1	237		124	3	9	57	
Kodiak 2	262		218	3	12	56	
Kodiak (Winter)		214	173	2	8	32	
PWS 1	209		111	3	7	36	
PWS 2	251		160	4	8	11	
Sitka 1	239	221		4	11	49	weights not taken
Sitka 2	233	220	121	3	9	50	
Togiak 1		305	413	6	12	49	
Togiak 2		285	334	4	15	50	
						613	

Table 4. Aggregate (sum across sample years) age frequency distribution, by sample area, for Pacific herring processed for fatty acid composition and elemental analysis, 2005-2007.

	Age (years)													
Sample Area	2	3	4	5	6	7	8	9	10	11	12	13	15	n
Bristol Bay			4	15	6	18	24	20	5	4	2		1	99
Dutch Harbor						1	2	9	8	2	2	1		25
Kamishak Bay		21	41	32	17	3								114
Kodiak Island	1	25	49	33	15	5	10	4	1	1	1			145
Kuskokwim Bay		2	16	13	5	9	20	16	1		2			84
PWS		6	5	14	13	6	3							47
Sitka		2	9	31	21	17	11	3	2	3				99
Totals	1	56	124	138	77	59	70	52	17	10	7	1	1	613

Table 5. Fatty acids used to discriminate herring stocks. The first column denotes fatty acids analyzed in 2001, bold font indicates fatty acids used to evaluate within-year differences under the first battery of tests. The second column denotes fatty acids analyzed in 2005, 2006, and 2007, bold font indicates those used to examine temporal stability in the second battery of tests.

Fatty acids analyzed in 2001	Fatty acids analyzed in 2005, 2006 and 2007
14:0	C12:0
14:1 (n-5)	**C14:0**
15:0	C14:1n5
15:1(n-5)	**C15:0**
16:0	C15:1n5
16:1(n-9)	C16:0
16:1(n-7)	**C16:1n7**
16:1(n-5)	**C17:0**
17:0	C17:1n7
17:1(n-7)	**C18:0**
18:0	C18:1n11
18:1(n-9)c&t	**C18:1n9c**
18:1(n-7)	**C18:1n7**
18:2(n-6)c	C18:2n6t
18:2(n-6)t	**C18:2n6c**
18:3(n-6)	**C18:3n6**
18:3(n-3)	**C20:0**
18:4(n-3)	**C18:3n3**
20:0	C20:1n11
20:1(n-11)	C20:1n9
20:1(n-9)	C18:4n3
20:1(n-7)	C20:2n6
20:2(n-9)	**C20:3n6**
20:2(n-6)	C22:0
20:3(n-6)	C20:3n3
20:4(n-6)	C20:4n6
20:3(n-3)	C22:1n11
20:4(n-3)	C22:1n9
20:5(n-3)	C20:4n3
22:0	**C20:5n3**
22:1(n-11)	C22:2n6
22:1(n-9)	C24:0
22:2(n-6)	C22:4n6
22:5(n-3)	C22:5n6
22:4(n-6)	C24:1n9
22:5(n-6)	**C22:5n3**
24:0	**C22:6n3**
22:6(n-3)	
24:1(n-9)	

Table 6. Confusion matrices for regional comparisons of herring fatty acids, values in cells depict the percentage of known samples assigned to a given region during cross-validation. The known region is identified by rows and the predicted region in columns. Column labeled "n" gives the number of fish classified.

		2001 Predicted Region			
Known Region	Bering Sea	NGA	SEAK		n
Bering Sea	94.4	3.6	0		18
NGA	0	99.2	0.8		134
SEAK	0	0	100		18
		2005 Predicted Region			
Known Region	Bering Sea	NGA	SEAK		n
Bering Sea	89.6	8.3	2.1		96
NGA	4.5	83.3	12.1		132
SEAK	0	12.5	87.5		40
		2006 Predicted Region			
Known Region	Bering Sea	NGA	SEAK	Aleutian Islands	n
Bering Sea	95.5	0	3.4	1.1	89
NGA	3.3	84.2	12.5	0	120
SEAK	0	6.6	90.0	3.3	60
Aleutian Is.	0	0	0	100	30

Table 7. ANOSIM R statistics for comparisons of fatty acid compositions between regions. * = 0.05 > P > 0.01; ** = 0.01 > P > 0.001; *** = P < 0.001.

	2001		
	NGA	SEAK	Bering Sea
NGA			
SEAK	0.149		
Bering	0.799***	0.813***	
	2005		
NGA			
SEAK	0.177***		
Bering	0.295***	0.453***	
	2006		
NGA			
SEAK	0.187***		
Bering	0.368***	0.391***	
Aleutian Islands	0.202***	0.136**	0.426***

Table 8. Confusion matrix for areas in the NGA region based on DFA's for 2001, 2005, and 2006. Values in cells depict the percentage of known samples assigned to a given region during cross-validation. The known area is identified by rows and predicted area in columns. Column labeled "n" gives the number of fish classified.

		2001 Predicted Region		
Known Region	Kamishak	Kodiak	PWS	n
Kamishak	84.8	15.2	0	33
Kodiak	8.0	88.0	4.0	25
PWS	2.7	6.7	90.5	74
		2005 Predicted Region		
Known Region	Kamishak	Kodiak	PWS	n
Kamishak	63.0	22.2	14.8	54
Kodiak	20.4	74.1	5.6	54
PWS	12.5	8.3	79.2	24
		2006 Predicted Region		
Known Region	Kamishak	Kodiak		n
Kamishak	78.3	21.7		60
Kodiak	8.3	91.7		60

Table 9. Matrix of ANOSIM R statistics comparing the fatty acid compositions of herring collected in the NGA in 2001, 2005, and 2006. * = 0.05 > P > 0.01; ** = 0.01 > P > 0.001; *** = P < 0.001.

2001		
	PWS	Kamishak
Kamishak	0.331***	
Kodiak	0.319***	0.173***
2005		
	PWS	Kamishak
Kamishak	0.055	
Kodiak	0.252***	0.067**
2006		
	Kodiak	
Kamishak	0.345***	

Table 10. Confusion matrix for Bering Sea areas in 2005 and 2006. Values in cells depict the percentage of known samples assigned to a given area during cross-validation. The known area is identified by rows and predicted area in columns. Column labeled "n" gives the number of fish classified.

	2005		
	Predicted Region		
Known Region	Bristol Bay	Kuskokwim Bay	n
Bristol Bay	87.8	12.2	41
Kuskokwim Bay	21.8	78.2	55

	2006		
	Predicted Region		
Known Region	Bristol Bay	Kuskokwim Bay	
Bristol Bay	93.2	6.8	59
Kuskokwim Bay	26.7	73.3	30

Table 11. Confusion matrix for comparisons made between sample sites within areas in 2005 and 2006. Values in cells are the percentage of observations correctly assigned to sites collected from a given area. Note only two sites were collected in a given year.

		Percentage Correctly Identified	
Area	Site	2005	2006
Sitka	Sitka Sound	100	93.3
	Hoonah Sound	100	100
Kuskokwim Bay	Goodnews Bay	92.6	a
	Toksook/Nelson	89.3	
Kodiak	Kiliuda Bay	88.9	90.0
	Uganik Bay	96.3	93.3
Kamishak Bay	Chenik/Nordyke	74.0	46.7
	Iniskin/Oil Bay	74.0	63.3
Bristol Bay	Hagemeister	33.0	66.7
	Nunavachak	55.0	69.0

[a]Sample not viable.

Table 12. Confusion matrices for models comparing the fatty acid compositions of herring collected from different regions in one year with those of herring collected in other years.

2001 Predictive DFA model

	Predicted Region			
Known Region	Bering Sea	NGA	SEAK	n
2005 Bering Sea	0	86.5	13.5	96
2005 NGA	0	67.4	32.6	132
2005 SEAK	0	47.5	52.5	40
2006 Bering Sea	0	100.0	0.0	89
2006 NGA	0	88.3	11.7	120
2006 SEAK	0	53.3	46.7	60
2006 Aleutian Is.	0	96.7	3.3	30
2007 NGA winter	0	56.7	42.3	30

2005 Predictive DFA model

	Predicted Region			
Known Region	Bering Sea	NGA	SEAK	n
2001 Bering Sea	22.2	0.0	77.8	18
2001 NGA	11.9	0.0	88.1	132
2001 SEAK	0.0	0.0	100.0	18
2006 Bering Sea	96.6	3.4	0.0	89
2006 NGA	3.3	83.3	13.3	120
2006 SEAK	5.0	53.3	41.7	60
2006 Aleutian Is.	16.7	83.3	0.0	30
2007 NGA winter	0.0	100.0	0.0	30

2006 Predictive DFA model

	Predicted Region				
Known Region	Bering Sea	NGA	SEAK	Aleut. Is.	n
2001 Bering Sea	100.0	0.0	0.0	0.0	18
2001 NGA	44.8	21.6	32.8	0.7	132
2001 SEAK	5.6	27.8	66.7	0.0	18
2005 Bering Sea	81.3	4.2	14.6	0.0	96
2005 NGA	3.0	67.4	18.9	10.6	132
2005 SEAK	2.5	27.5	67.5	2.5	40
2007 NGA winter	3.3	40.0	0.0	56.7	30

Table 13. Matrix of ANOSIM R statistics comparing the similarity between regions, across years (also see Figure 2). All compositions were significantly different (P < 0.01).

	SEAK 01	NGA 01	BerSea 01	BerSea 05	NGA 05	SEAK 05	BerSea 06	Aleut I 06	NGA 06	SEAK 06
SEAK 01										
NGA 01	0.267									
Bering Sea 01	0.735	0.777								
BerSea 05	.956	.864	.991							
NGA 05	.815	.766	.991	.251						
SEAK 05	.659	.806	.927	.474	.249					
BerSea 06	.973	.874	.991	.317	.463	.669				
Aleut I 06	.955	.866	.945	.618	.341	.586	.549			
NGA 06	.680	.730	.973	.363	.176	.301	.355	.185		
SEAK 06	.690	.825	.919	.405	.324	.165	.389	.266	.210	
NGA winter	.874	.953	.890	.948	.893	.798	.952	.808	.761	.789

Table 14. Confusion matrices resulting from fitting herring collected from areas in the NGA in one year to DFA models constructed from herring collected in other years. Values show the percentage of samples assigned to each area. *PWS was not sampled in 2006.

2001 Predictive DFA model

	Predicted Area			
Known Area	Kamishak	Kodiak	PWS	n
2005 Kamishak	92.6	3.7	3.7	54
2005 Kodiak	79.6	9.3	11.1	54
2005 PWS	95.8	4.2	0	24
2006 Kamishak	96.7	1.7	1.7	60
2006 Kodiak	85.0	8.3	6.7	60
2007 Kodiak	93.3	3.3	3.3	30

2005 Predictive DFA model

	Predicted Area			
Known Area	Kamishak	Kodiak	PWS	n
2001 Kamishak	27.3	69.7	3.0	33
2001 Kodiak	11.1	85.2	3.7	27
2001 PWS	1.4	90.5	8.1	74
2006 Kamishak	81.7	15.0	3.3	60
2006 Kodiak	38.3	53.3	8.3	60
2007 Kodiak	30.0	0	70.0	30

2006 Predictive DFA model

	Predicted Area			
Known Area	Kamishak	Kodiak	PWS*	n
2001 Kamishak	12.1	87.9	NS	33
2001 Kodiak	25.9	74.1	NS	27
2001 PWS	6.8	93.2	NS	74
2005 Kamishak	31.5	68.5	NS	54
2005 Kodiak	7.4	92.6	NS	54
2005 PWS	25.0	75.0	NS	24
2007 Kodiak	86.7	13.3	NS	30

Table 15. Confusion matrices resulting from fitting herring collected from areas in the Bering Sea in one year to DFA models constructed from herring collected in other years. Values show the percentage of samples assigned to each area.

2005 Predictive DFA model

	Predicted Area		
Known Area	Kuskokwim Bay	Bristol Bay	n
2001 Bristol Bay	0	100.0	18
2006 Bristol Bay	35.6	64.4	59
2006 Kuskokwim Bay	86.7	13.3	30

2006 Predictive DFA model

	Predicted Area		
Known Area	Kuskokwim Bay	Bristol Bay	n
2001 Bristol Bay	66.7	33.3	18
2005 Bristol Bay	34.1	65.9	41
2005 Kuskokwim Bay	14.5	85.5	55

Table 16. Confusion matrices resulting from fitting herring collected from sites in SEAK in one year to DFA models constructed from herring collected in different years. Values show the percentage of samples assigned to each site.

2005 Predictive DFA model

	Predicted Site	
Known Site	Hoonah Sound	Sitka Sound
2001 Sitka Sound	22.2	77.8
2006 Hoonah Sound	100.0	0.0
2006 Sitka Sound	16.7	83.3

2006 Predictive DFA model

	Predicted Site	
Known Site	Hoonah Sound	Sitka Sound
2001 Sitka Sound	5.6	94.4
2005 Hoonah Sound	80.0	20.0
2005 Sitka Sound	5.0	95.0

Table 17. Confusion matrix for identities of otoliths based on discriminant functions derived from the composition of otolith cores.

From \ To	Aleutian	Bering Sea	NGA	SEAK	Total	% Correct
Aleutian	0	4	10	2	16	0.0%
Bering Sea	0	24	33	0	57	42.1%
NGA	0	20	43	4	67	64.2%
SEAK	0	9	20	6	35	17.1%
Total	0	57	106	12	175	41.7%

Table 18. Confusion matrix for identities of otoliths based on discriminant functions derived from the composition of otolith edges.

From \ To	Aleutian	Bering Sea	NGA	SEAK	Total	% Correct
Aleutian	5	8	7	2	22	22.7%
Bering Sea	2	30	40	2	74	40.5%
NGA	2	20	68	2	92	73.9%
SEAK	0	15	29	2	46	4.3%
Total	9	73	144	8	234	44.9%

Table 19. ANOSIM comparisons of herring otolith core chemistries sampled from four regions in Alaska in 2006. Values in the table are R's, which indicate centroid distances. R values were not significantly different from zero.

	Bering Sea	Aleutian	NGA	SEAK
Bering Sea				
Aleutian	0.08314			
NGA	-0.00318	0.05087		
SEAK	0.01560	0.01331	-0.00529	

Table 20. ANOSIM comparisons of herring otolith edge chemistries sampled from four regions in Alaska in 2006. Values in the table are R's, which indicate centroid distances. R values in bold were significantly different from zero.

	Aleutian	Bering Sea	NGA	SEAK
Aleutian				
Bering Sea	0.06993			
NGA	**0.22870**	**0.04401**		
SEAK	0.02268	0.01600	**0.09134**	

Table 21. Confusion matrix for identities of otoliths collected in Southeast Alaska (SEAK) based on discriminant functions derived from the composition of otolith cores.

From \ To	Hoonah Sound	Sitka Sound	Total	% Correct
Hoonah Sound	15	4	19	78.9%
Sitka Sound	8	8	16	50.0%
Total	23	12	35	65.7%

Table 22. Confusion matrix for identities of otoliths collected in Southeast Alaska (SEAK) based on discriminant functions derived from the composition of otolith edges.

From \ To	Hoonah Sound	Sitka Sound	Total	% Correct
Hoonah Sound	22	3	25	88.0%
Sitka Sound	13	8	21	38.1%
Total	35	11	46	65.2%

Table 23. ANOSIM comparisons of otolith core chemistries from herring collected at ten sites in 2006. Values in the table are R's, which indicate centroid distances. R in bold are significantly different from zero.

	Nelson Island	Iliulink Bay	Oil Bay	Nordyke Island	Uganik Bay	Kiliuda Bay	Sitka Sound	Hoonah Sound	Nunavachak Bay	Hagemeister Island
Nelson Island										
Iliulink Bay	0.0177									
Oil Bay	-0.0312	0.0065								
Nordyke Island	-0.0103	0.0371	-0.0174							
Uganik Bay	0.0067	-0.0356	0.0458	0.0626						
Kiliuda Bay	-0.0188	-0.0300	-0.0008	-0.0049	-0.0353					
Sitka Sound	**0.0958**	-0.0123	-0.0077	0.0296	0.0493	0.0380				
Hoonah Sound	-0.0361	-0.0015	0.0051	0.0178	0.0022	-0.0189	0.0690			
Nunavachak Bay	**0.1377**	0.0225	0.0551	**0.1013**	0.0847	0.0827	-0.0213	**0.1061**		
Hagemeister Island	0.0204	0.0544	-0.0060	-0.0114	0.0966	0.0371	-0.0011	0.0690	0.0550	

Table 24. ANOSIM comparisons of otolith edge chemistries from herring collected at ten sites in 2006. Values in the table are R's, which indicate centroid distances. R's in bold are significantly different from zero.

	Iliulink Bay	Nelson Island	Oil Bay	Kiliuda Bay	Nordyke Island	Uganik Bay	Hoonah Sound	Sitka Sound	Hagemeister Island	Nunavachak Bay
Iliulink Bay										
Nelson Island	**0.0885**									
Oil Bay	**0.2283**	0.0190								
Kiliuda Bay	**0.1013**	-0.0244	0.0510							
Nordyke Island	**0.1921**	0.0175	-0.0081	0.0514						
Uganik Bay	**0.1951**	0.0160	-0.0073	0.0494	-0.0152					
Hoonah Sound	0.0096	0.0378	**0.1195**	0.0298	**0.0925**	**0.1131**				
Sitka Sound	0.0494	-0.0075	0.0477	0.0223	-0.0034	0.0169	0.0188			
Hagemeister Island	**0.0613**	-0.0353	0.0254	-0.0183	0.0315	0.0213	0.0218	0.0015		
Nunavachak Bay	-0.0109	**0.0806**	**0.1906**	**0.0704**	**0.1643**	**0.1677**	-0.0178	0.0600	**0.0543**	

Table 25. Confusion matrix for identities of otoliths sampled from four spawning aggregations in the North Gulf of Alaska (NGA) in 2006, based on discriminant functions derived from the composition of otolith cores. The last row provides the result of fitting the Kodiak (Winter) sample to this DFA in an attempt to determine the spawning stock of origin for herring aggregated in Uganik Bay, Kodiak Island, in November 2007.

From \ To	Kiliuda Bay	Nordyke Island	Oil Bay	Uganik Bay	Total	% Correct
Kodiak- Kiliuda Bay	2	4	4	4	14	14.3%
Kamishak- Nordyke Island	3	3	9	3	18	16.7%
Kamishak- Oil Bay	0	4	13	3	20	65.0%
Kodiak-Uganik Bay	0	1	7	7	15	46.7%
Total	5	12	33	17	67	37.3%
Kodiak-Uganik (Winter '07)	*1*	*4*	*12*	*6*	*23*	*26.1%*

Table 26. Confusion matrix for identities of otoliths sampled from spawning herring in the North Gulf of Alaska (NGA) in 2006, based on discriminant functions derived from the composition of otolith edges. The last row provides the result of fitting the Kodiak (Winter) sample to this DFA in an attempt to determine the spawning stock of origin for herring aggregated in Uganik Bay, Kodiak Island, in November 2007.

From \ To	Kiliuda Bay	Nordyke Island	Oil Bay	Uganik Bay	Total	% Correct
Kodiak- Kiliuda Bay	17	1	5	2	25	68.0%
Kamishak- Nordyke Island	7	4	8	2	21	19.0%
Kamishak- Oil Bay	6	1	13	5	25	52.0%
Kodiak-Uganik Bay	5	1	6	9	21	42.9%
Total	35	7	32	18	92	46.7%
Kodiak-Uganik (Winter '07)	*8*	*0*	*5*	*11*	*24*	*45.8%*

Table 27. ANOSIM comparisons of otolith core chemistries for four North Gulf of Alaska (NGA) spawning aggregations sampled in Spring 2006 and one overwintering aggregation in November 2007. Values in the table are R's, which indicate centroid distances. R in bold is significantly different from zero.

	Oil Bay	Nordyke Island	2007-Kodiak (Winter)	Uganik Bay	Kiliuda Bay
Oil Bay					
Nordyke Island	-0.01743				
2007-Kodiak (Winter)	-0.00149	0.01494			
Uganik Bay	0.04583	0.06256	**0.13215**		
Kiliuda Bay	-0.00084	-0.00494	0.06229	-0.03533	

Table 28. ANOSIM comparisons of otolith edge chemistries for four North Gulf of Alaska (NGA) spawning aggregations sampled in Spring 2006 and one overwintering aggregation in November 2007. Values in the table are R's, which indicate centroid distances. R in bold is significantly different from zero.

	Oil Bay	Kiliuda Bay	Nordyke Island	Uganik Bay	2007-Kodiak (Winter)
Oil Bay					
Kiliuda Bay	**0.05103**				
Nordyke Island	-0.00813	0.05136			
Uganik Bay	-0.00734	0.04937	-0.01525		
2007-Kodiak (Winter)	-0.01502	0.04404	-0.01769	-0.03010	

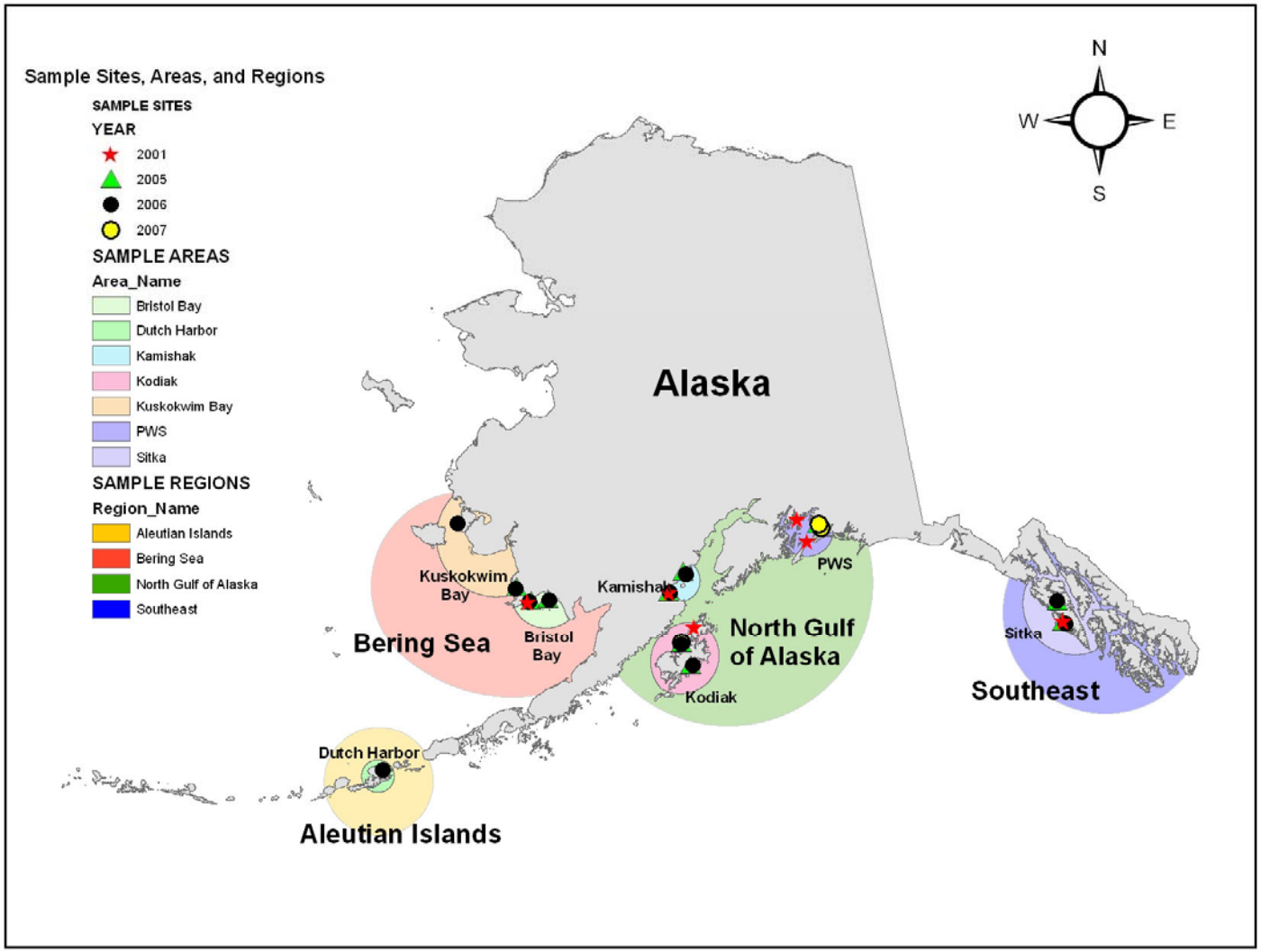


Figure 1. Map of Alaska illustrating the four regions, seven areas, and thirteen sample sites where Pacific herring were collected in 2005-2007 to evaluate stock structure. Sites sampled during a 2001 pilot study (red stars) are also shown for reference.

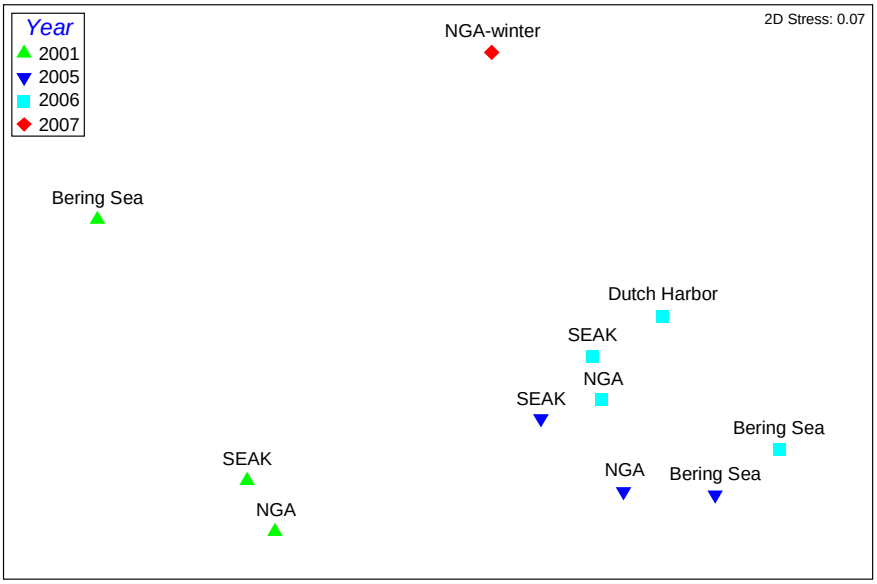
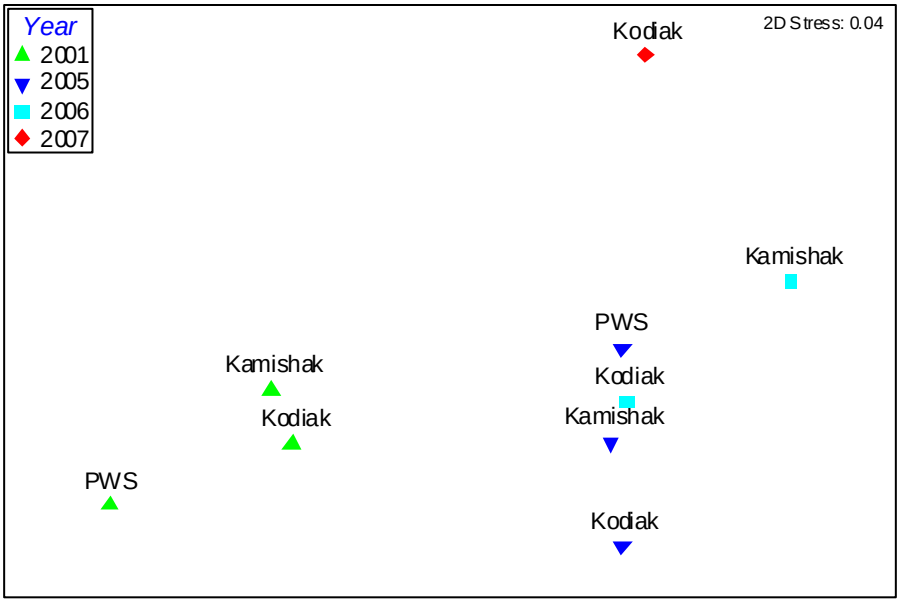


Figure 2. Second stage nMDS plot for herring collected from different regions in 2001, 2005, 2006, and 2007. Note that most of the distance among regional centroids can be accounted for by temporal rather than spatial factors. See Table 13 for associated R statistics.



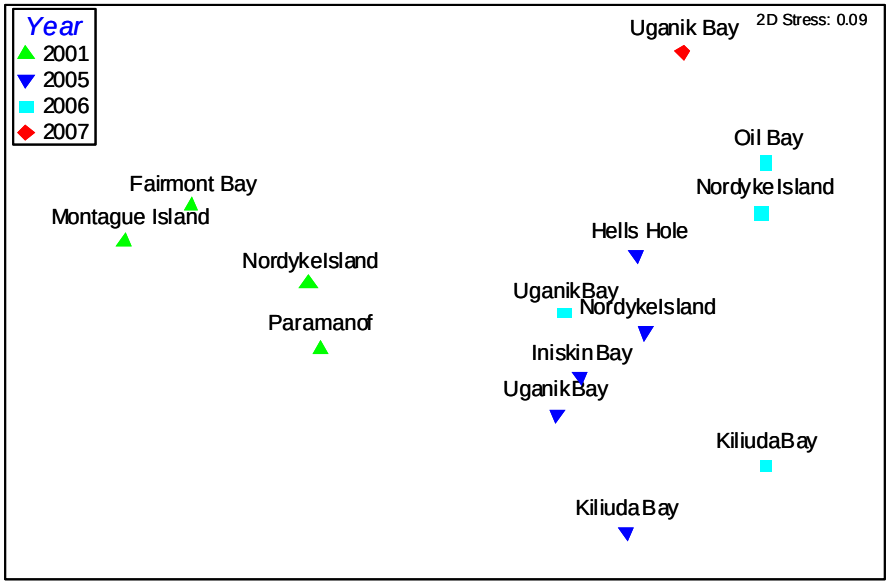


Figure 3. Second stage nMDS plots for herring collected from areas in the NGA in 2001, 2005, and 2006 (top panel), and individual sample sites within areas in the NGA (bottom panel).

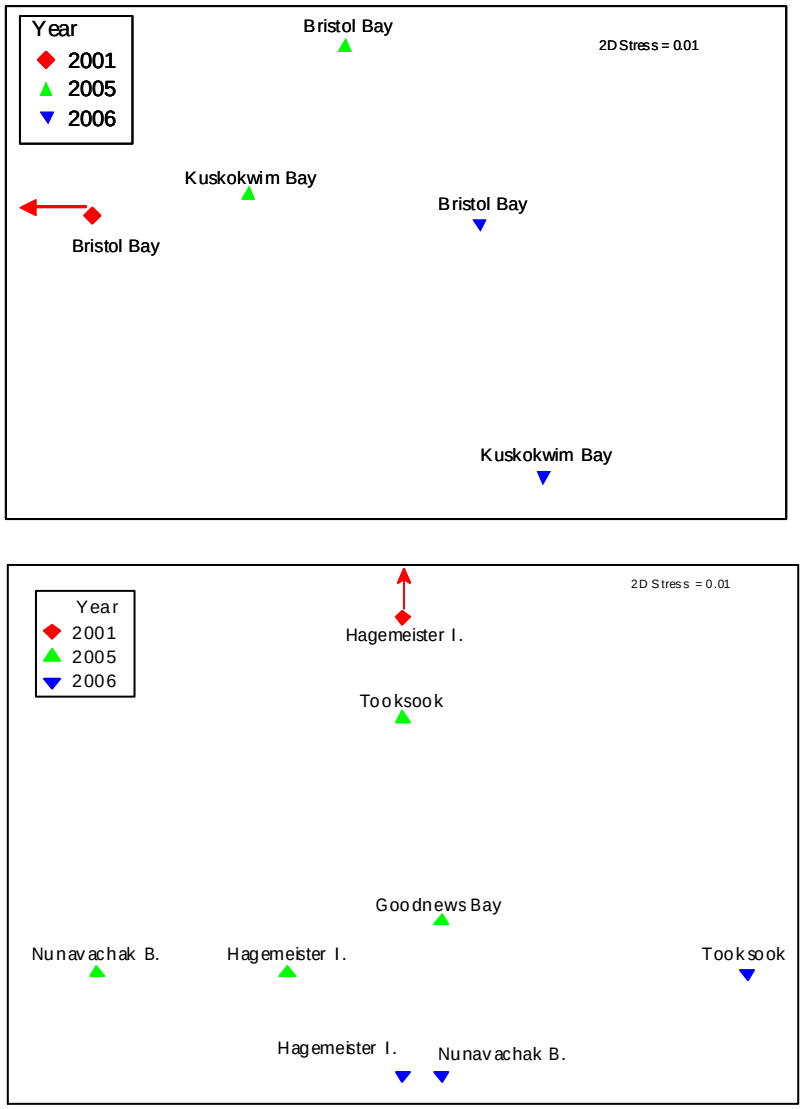
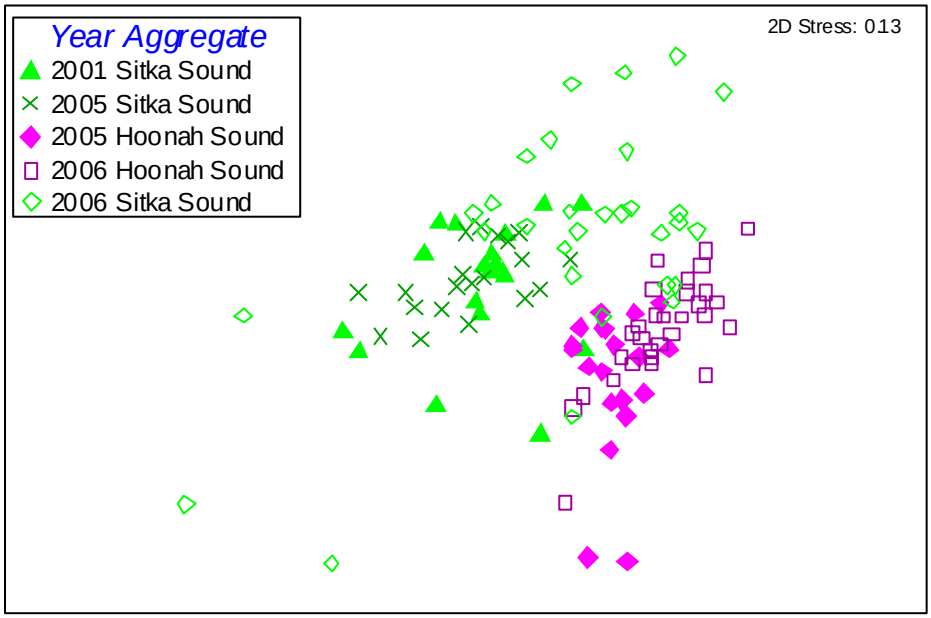


Figure 4. Second stage nMDS plots of herring collected from areas in the Bering Sea in 2001, 2005, and 2006 (top panel), and individual sample sites from areas in the Bering Sea (bottom panel). Note the location of the 2001 Bering Sea samples is off the scale in the direction shown on the graphs.



	Sitka 01	Sitka 05	Sitka 06	Hoonah 05
Sitka 05	0.530			
Sitka 06	0.302	0.683		
Hoonah 05	0.683	0.831	0.382	
Hoonah 06	.885	.907	0.322	.280

Figure 5. nMDS plot and R statistics comparing fatty acid compositions of herring collected in 2001, 2005, and 2006 at the Sitka and Hoonah sample sites in SEAK. All compositions were significantly different (P < 0.001).

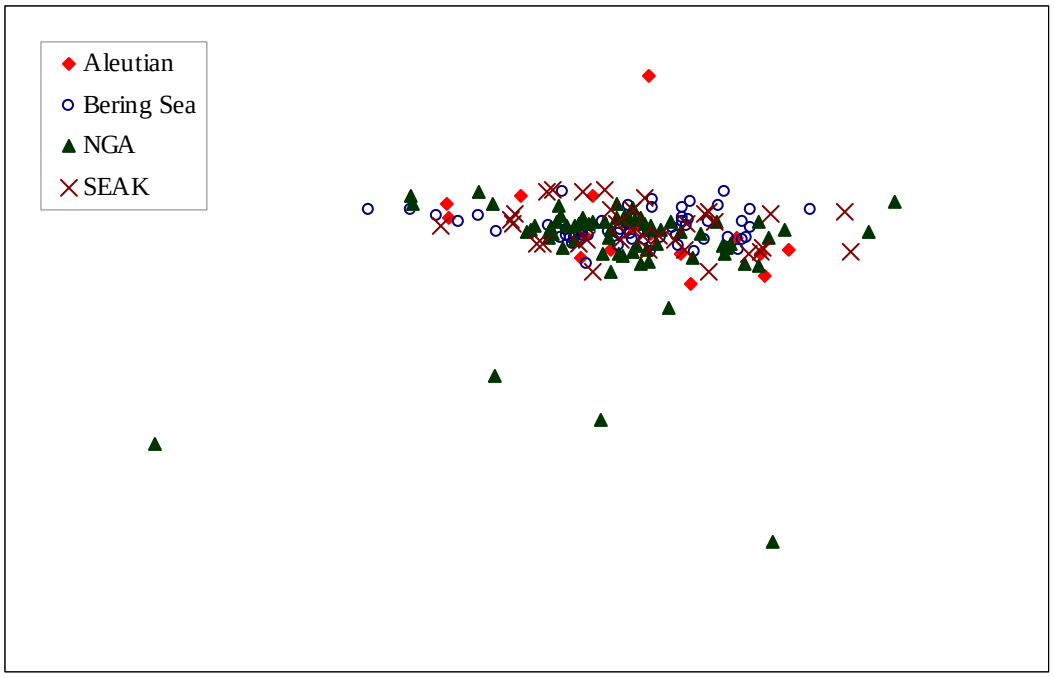


Figure 6. nMDS plot depicting spatial relationships between herring from different regions based on the elemental composition of their otolith cores. Kruskal stress = 0.01.

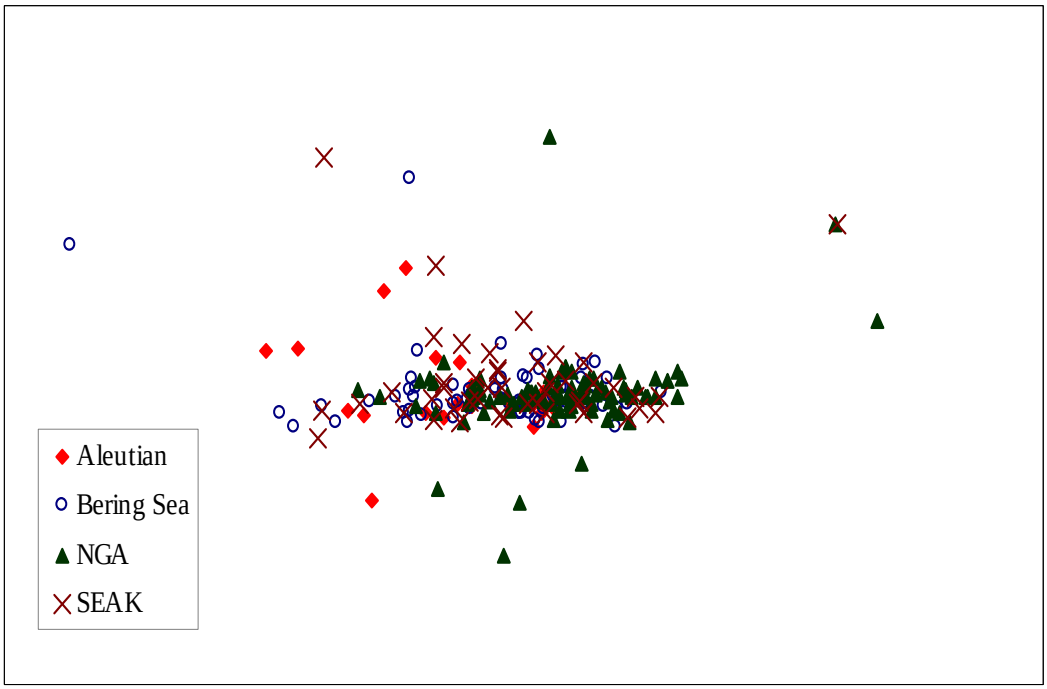


Figure 7. nMDS plot depicting spatial relationships between herring from different regions based on the elemental compositions of their otolith edges. Kruskal stress = 0.01.

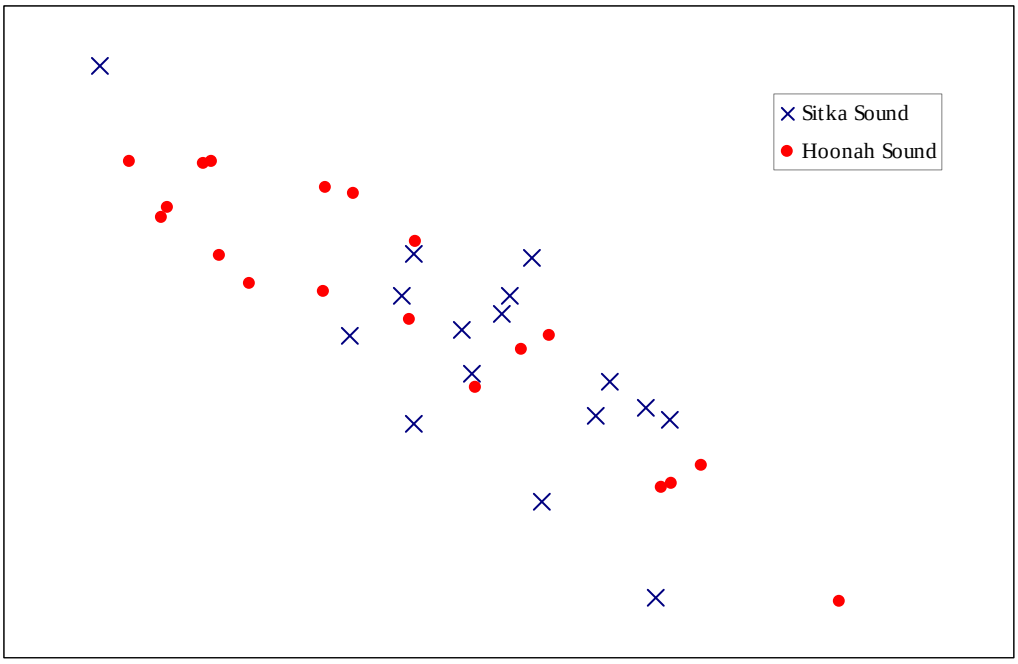


Figure 8. nMDS plot depicting spatial relationships between fish from different sites in Southeast Alaska (SEAK) based on the elemental compositions of otolith cores. Kruskal stress = 0.017.

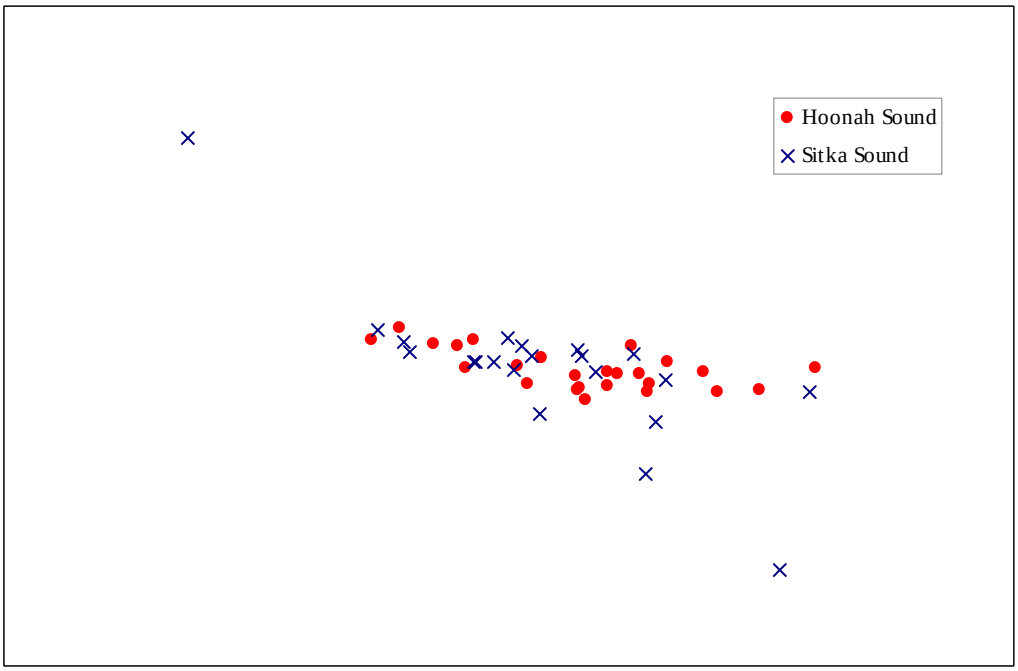


Figure 9. nMDS plot depicting spatial relationships between fish from different sites in Southeast Alaska (SEAK) based on the elemental compositions of otolith edges. Kruskal stress = 0.027.

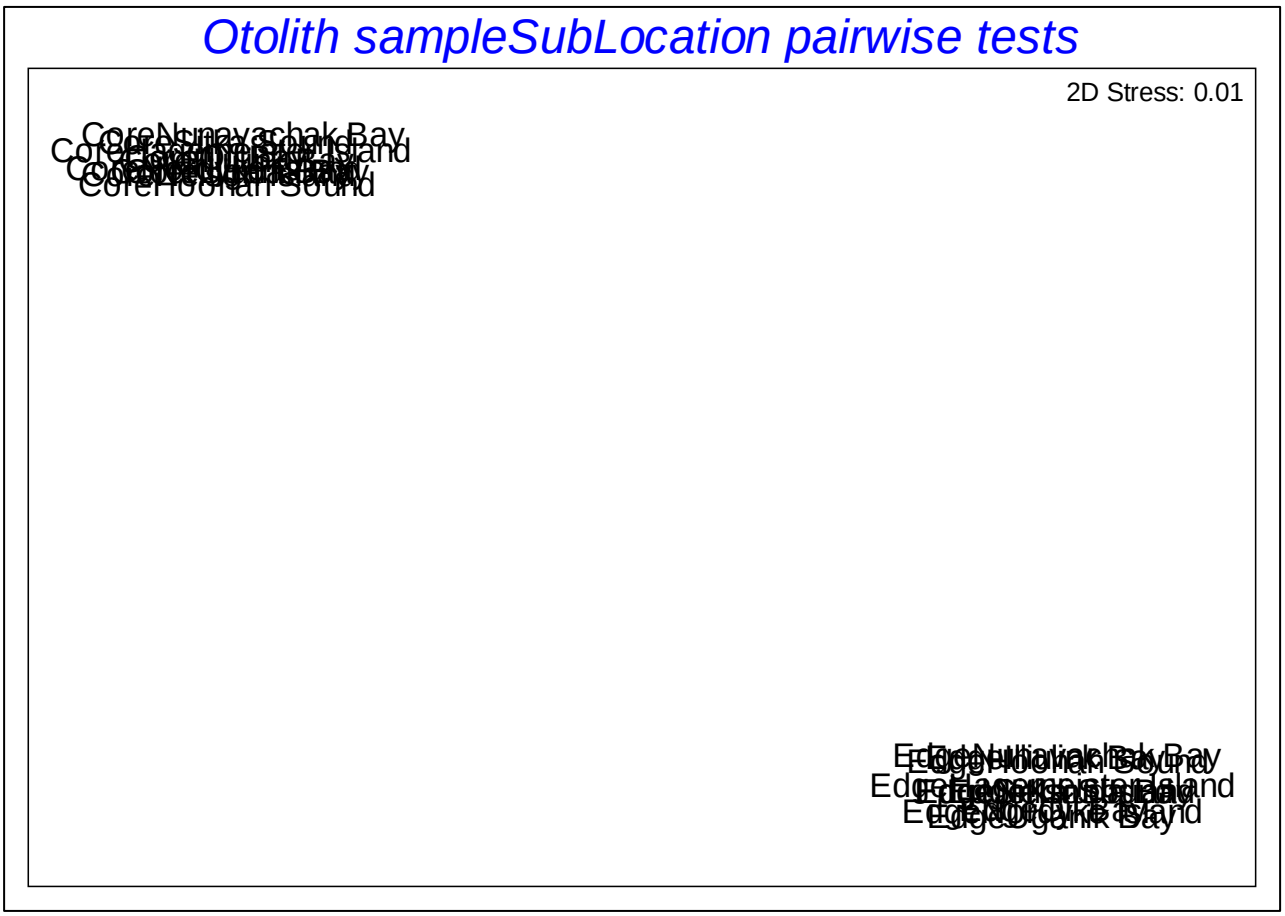


Figure 10. Second stage nMDS plot showing the centroid locations for otolith chemistries of herring sampled from ten different sites in Alaska in 2006. Element:Ca ratios are based on analysis of otolith cores (upper left) and edges (lower right). Median R between core and edge is 0.60. Median R between sites within core comparisons is 0.017, median R between sites within edge comparisons is 0.05.

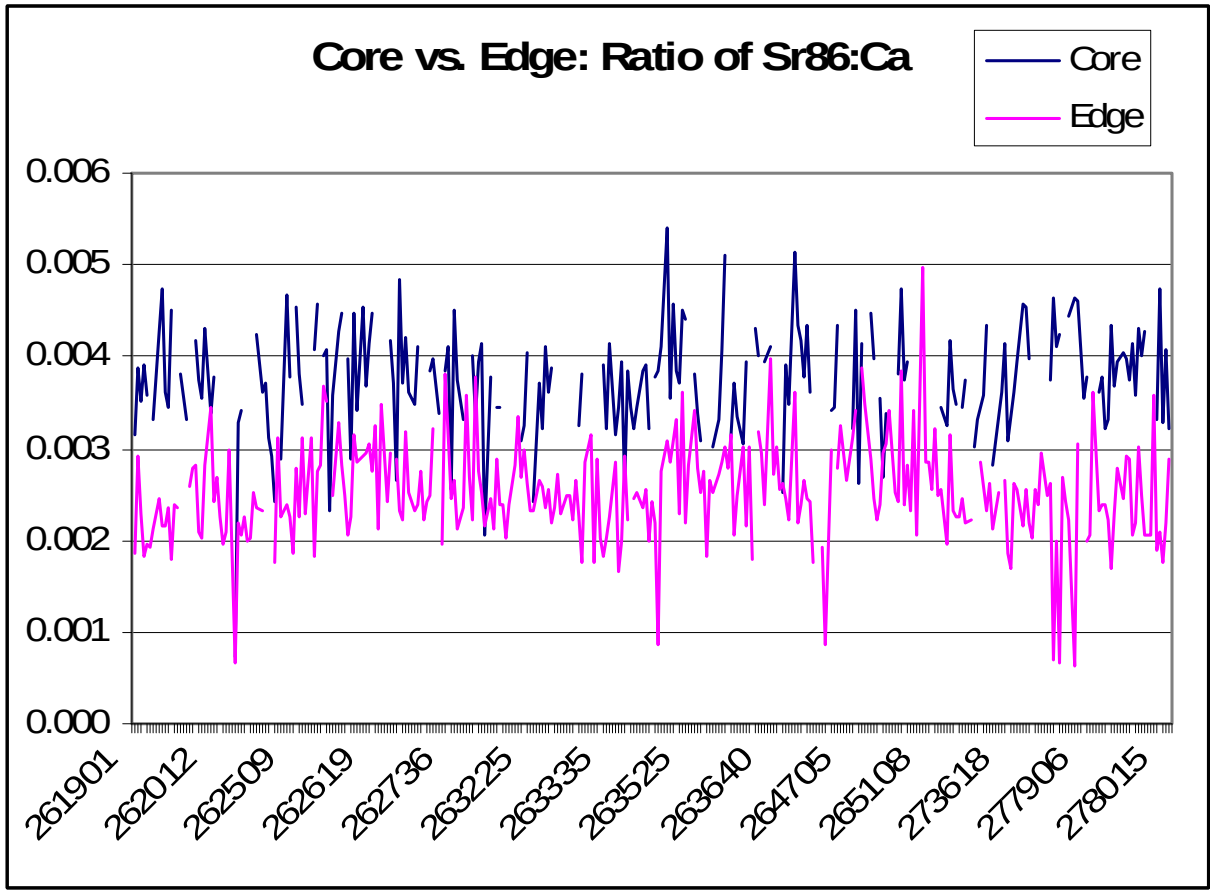


Figure 11. Plot of Sr:Ca ratios for otolith core and edge data. Each tick on the x-axis represents an individual fish sampled (n = 283) from ten spawning aggregations across Alaska during the Spring of 2006. Values on x-axis refer to specimen identification numbers.

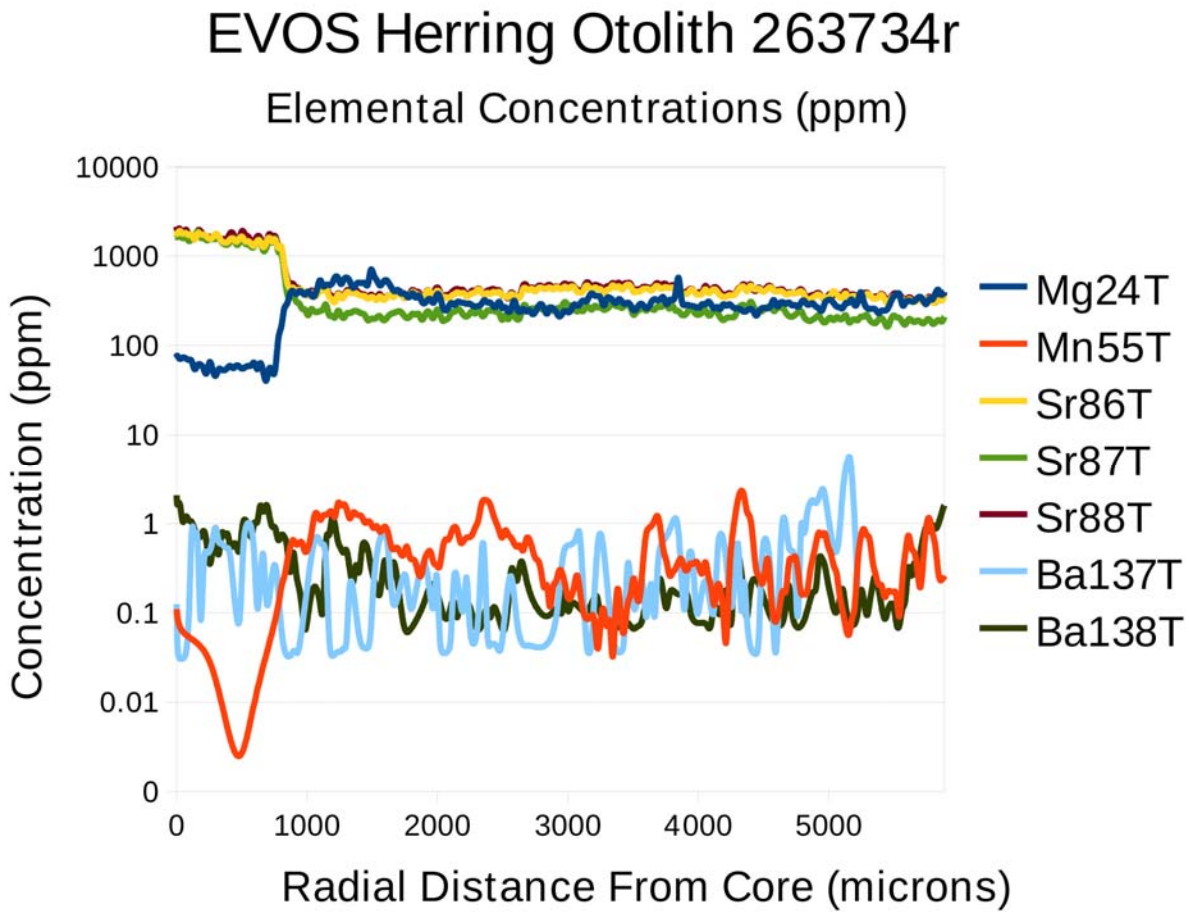


Figure 12. Concentration (ppm) of elements measured by LA-ICPMS from the core to the edge of an otolith collected from a Pacific herring in Hoonah Sound, Alaska, 2006. Note the elevated level of Sr and depressed level of Mg in the core region.

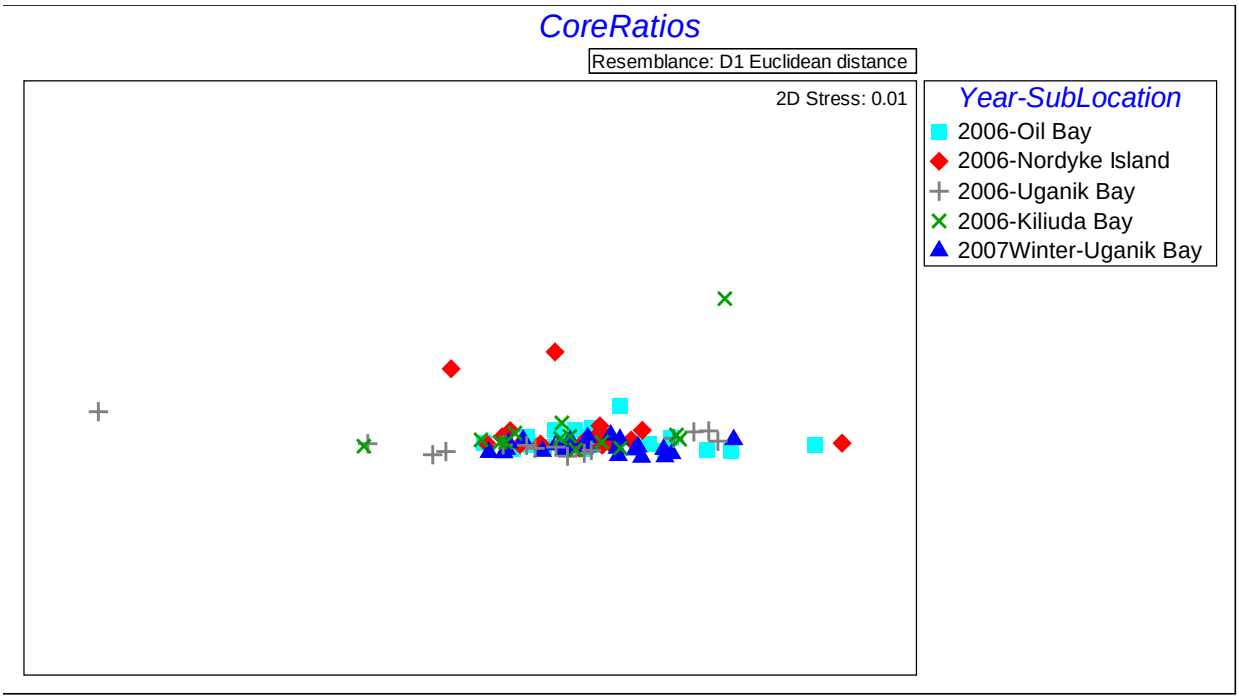


Figure 13. nMDS plot depicting spatial relationships between otolith core chemistries for four North Gulf of Alaska (NGA) spawning aggregations sampled in Spring 2006 and one overwintering aggregation in Winter 2007.

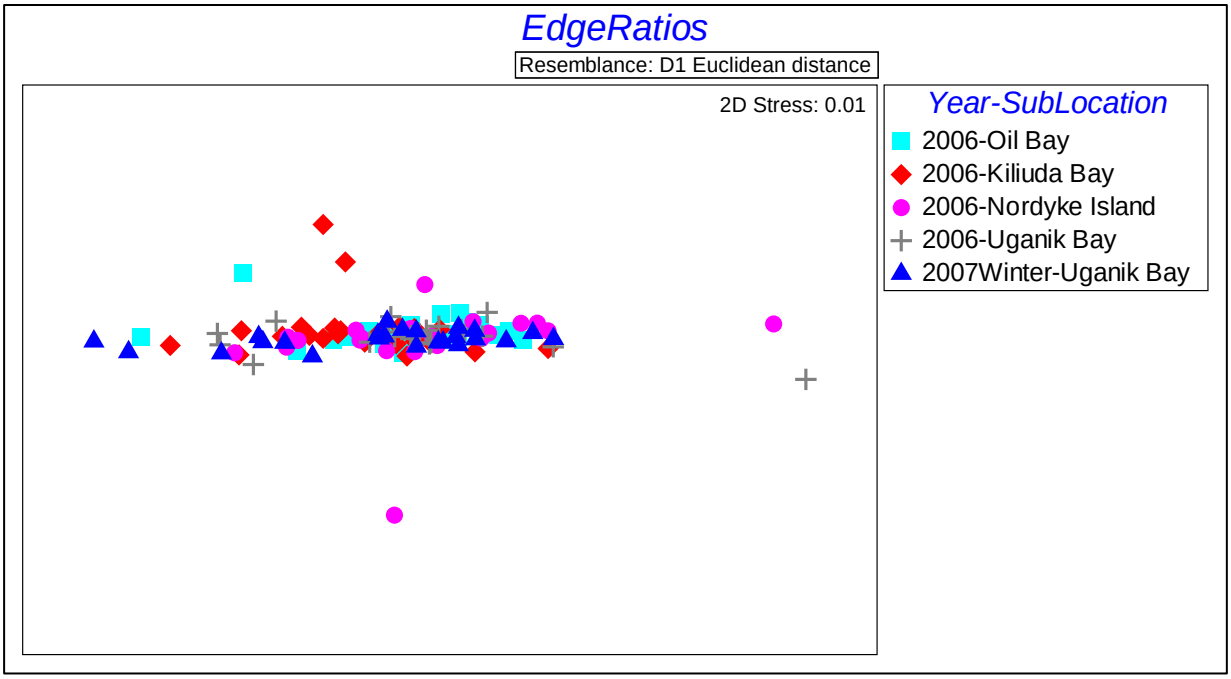


Figure 14. nMDS plot depicting spatial relationships between otolith edge chemistries for four North Gulf of Alaska (NGA) spawning aggregations sampled in Spring 2006 and one overwintering aggregation in Winter 2007.

LITERATURE CITED

Ackman, R.G., P.J. Ke, W.A. MacCallum, and D.R. Adams. 1969. Newfoundland capelin lipids: fatty acid composition and alterations during frozen storage. Journal Fisheries Research Board of Canada 26(8):2037-2060.

Agnisola, C., D.J. McKenzie, E.W. Taylor, C.L. Bolis and B. Tota. 1996. Cardiac performance in relation to oxygen supply varies with dietary lipid composition in sturgeon. Am. J. Physiol. 271:417-425.

Aidos, I., S. Lourenco, A. Van Der Padt, J.B. Luten, and R.M. Boom. 2002. Stability of crude herring oil produced from fresh byproducts: Influence of temperature during storage. Journal of Food Science 67(9):3314-3320.

Anelich, L.E., L.C. Hoffman, and M.J. Swanepoel. 2001. The influence of packaging methodology on the microbiological and fatty acid profiles of refrigerated African catfish fillets. Journal of Applied Microbiology 91:22-28.

Ayvazian, S.G., T.P. Bastow, J.S. Edmonds, J. How, and G.B. Nowara. 2004. Stock structure of Australian herring (*Arripis georgiana*) in Southwestern Australia. Fisheries Research 67: 39-53.

Barros P., and J.C. Holst. 1995. Identification of geographic origin of Norwegian spring-spawning herring (*Clupea harengus* L.) based on measurements of scale annuli. ICES J. Mar. Sci. 52: 863-872.

Beacham, T.D., J.F. Schweigert, C. MacConnachie, K.D. Le, K. Labaree, and K.M. Miller. 2002. Population structure of herring (*Clupea pallasii*) in British Columbia determined by microsatellites, with comparisons to Southeast Alaska and California. Canadian Science Advisory Secretariat Research Document 2002/109.

Beckman, D.W., C.A. Wilson, F. Lorica, and J.M. Dean. 1990. Variability in incorporation of calcein as a fluorescent marker in fish otoliths. In N.C. Parker, A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, and G.A. Winans (eds.) Fish Marking Techniques, American Fisheries Society Symposium 7. 879 pp.

Begg, G.A., K.D. Friedland, and J.B. Pearce. 1999a. Stock identification and its role in stock assessment and fisheries management: an overview. Fisheries Research 43:1-8.

Begg, G.A., J.A. Hare, and D.D. Sheehan. 1999b. The role of life history parameters as indicators of stock structure. Fisheries Research 43:141-163.

Begg, G.A. and J.R. Waldman. 1999. An holistic approach to fisher stock identification. Fisheries Research 43:35-44.

Bell, J.G., J.R. Dick, A.H. McVicar, J.R. Sargent and K.D. Thompson. 1993. Dietary sunflower, linseed and fish oils affect phospholipid fatty acid composition, development of cardiac lesions, phospholipase activity and eicosanoid production in Atlantic salmon (*Salmo salar*). Prostaglandins, Leukotrienes, Essential Fatty Acids 49:665-673.

Bembo, D.G., G.R. Carvalho, N. Cingolani, and T.J. Pitcher. 1996. Electrophoretic analysis of stock structure in Northern Mediterranean anchovies, *Engraulis encrasicolus*. ICES Journal of Marine Science 53:115-128.

Benson, A.J., S.P. Cox, and A. Springford. 2007. An evaluation of stock structure in Pacific herring. Paper presented at the 2007 PICES Meeting entitled- The changing North Pacific: Previous Patterns, Future Projections and Ecosystem Impacts.

Bentzen, P. 1998. Development of Mitochondrial DNA Markers and Use to Screen Prince William Sound Herring Populations for Genetic Differentiation. Appendix D *in* Seeb, J.E., S.E. Merkouris, L.W. Seeb, J.B. Olsen, P. Bentzen, and J.M. Wright. 1998. Genetic discrimination of Prince William Sound herring populations, *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Proje97165), Alaska Department of Fish and Game, Genetics Laboratory, Anchorage, Alaska.

Berghahn, R. and Karakiri, M. 1990. Experimental induction of biological tags in otoliths of 0-group plaice *Pleuronectes platessa* by starvation, temperature, and UV-B radiation. Marine Ecology Progress Series, 67: 227-233.

Bergenius, M.A.J., B.D. Mapstone, G.A. Begg, C.D. Murchie. 2005. The use of otolith chemistry to determine stock structure of three epinepheline serranid coral reef fishes on the Great Barrier Reef, Australia. Fisheries Research 72: 253-270.

Bickford, N. and Hannigan, R., 2005. Stock identification of Walleye (Sander vitreum) using otolith chemistry in the Eleven Point River, AR. North American Journal of Fisheries Management. 25: 1542-1549.

Brophy, D. and B.S. Danilowicz. 2002. Tracing populations of Atlantic herring in the Celtic and Baltic seas using otolith microstructure. ICES Journal of Marine Science 59(6):1305-1313.

Brophy, D., B.S. Danilowicz, and T.E. Jeffries. 2003. The detection of elements in larval otoliths from Atlantic herring using laser ablation ICP-MS. Journal of Fish Biology 63:990-1007.

Brophy, D., T.E. Jeffries, and B.S. Danilowicz. 2004. Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. Marine Biology 144:779-786.

Brophy, D., B.S. Danilowicz, and P.A. King. 2006. Spawning season fidelity in sympatric populations of Atlantic herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Sciences 63:607-616.

Brown, R.J. and K.P. Severin. 2009. Otolith chemistry analyses indicate water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not marine fish. Canadian Journal of Fisheries and Aquatic Sciences 66:1790-1808.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22(4):759-801.

Burbank, D.C. 1977. Circulation studies in Kachemak Bay and lower Cook Inlet. Vol. 3. Environmental studies of Kachemak Bay and lower Cook Inlet (Trasky, L.L., L.B. Flagg, and D.C. Burbank., eds.). Alaska Department of Fish and Game, Anchorage, AK, 207 p.

Caballero, M.J., A. Obach, G. Rosenlund, D. Montero, M. Gisvold, and M.S. Izquierdo. 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 214:253-271.

Cadrin, S.X., K.L. Bolles, W.J. Overholtz, M.P. Armstrong, and K.D. Friedland. 2005. Using multi-disciplinary stock identification to optimize morphometric discrimination of Atlantic herring spawning groups off New England. Theme Session on Multidisciplinary Approaches to the Identification of Stock Structure of Small Pelagics: Implications for Assessment and Sustainable Management (K). ICES CM 2004/K:09. 23 pp.

Campana, S.E. and J.D. Neilson. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci 42: 1014-1032.

Campana, S.E., J.A. Gagne, and J. Munro. 1987. Otolith microstructure of larval herring (Clupea harengus): Image or reality? Canadian Journal of Fisheries and Aquatic Sciences 44:1922-1929.

Campana, S.E. and J.A. Gagne. 1995. Cod stock discrimination using ICPMS elemental assays of otoliths. Pages 671-691 In: Secor D.H., J.M. Dean , S.E. Campana (eds), New developments in fish otolith research. University of South Carolina Press, Columbia.

Campana, S.E., J.A. Gagne, J.W. McLaren. 1995. Elemental fingerprinting of fish otoliths using ID-ICPMS. Mar. Ecol. Prog. Ser. 122: 115-120.

Campana, S.E., S.R. Thorrold, S.M. Jones, D.Guenther, M. Tubrett, H. Longerich, S. Jackson, N.M. Halden, J.M. Kalish, P. Piccoli, H. De Pontual, H. Troadec, J. Panfili, D.H. Secor. 1997. Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation

inductively coupled plasma mass spectrometry. Canadian Journal of Fisheries and Aquatic Sciences 54:2068-2079.

Campana, S. E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series, 188, 263-297.

Castell, J.D., L.D. Boston, R.J. Miller, and T. Kenchington. 1995. The potential identification of the geographic origin of lobster eggs from various wild stocks based on fatty acid composition. Can. J. Fish. Aquat. Sci. 52:1135-1140.

Chatelier, A., D.J. McKenzie, A. Prinet, R. Galois, J. Robin, J. Zamonino, and G. Claireaux. 2006. Associations between tissue fatty acid composition and physiological traits of performance and metabolism in the seabass (Dicentrarchus labrax). Journal of Experimental Biology 209:3429-3439.

Chittaro, P.M., J.D. Hogan, J. Gagnon, B.J. Fryer, and P.F. Sale. 2006. In situ experiment of ontogenetic variability in the otolith chemistry of Stegastes partitus. Marine Biology 149:1227-1235.

Christie, W. W. 2003. Lipid analysis: isolation, separation, identification, and structural analysis of lipids, 3rd edition. Oily Press, Bridgewater, England.

Clarke, K.R.; Warwick, R.M. 2001. Changes in marine communities: an approach to statistical analysis and interpretation. 2nd Edition. PRIMER-E: Plymouth, UK. 172 pp.

Clausen, L.W., Bekkevold, D., Hatfield, E.M.C. and Mosegaard, H. 2005. Application and validation of otolith microstructure as stock identifier in mixed Atlantic herring (*Clupea harengus*) stocks in the North Sea and Western Baltic. Theme Session on Multidisciplinary Approaches to the Identification of Stock Structure of Small Pelagics: Implications for Assessment and Sustainable Management (K). ICES CM 2005/K:05. 16 pp.

Cordier, M., G. Brichon, J-M. Weber, and G. Zwingelstein. 2002. Changes in the fatty acid composition of phospholipids in tissues of farmed sea bass (*Dicentrarchus labrax*) during an annual cycle. Roles of environmental temperature and salinity. Comparative Biochemistry and Physiology Part B 133:281-288.

Courtney, D.L., D.G. Mortensen, J.A. Orsi, and K. Munk. 2000. Origin of juvenile Pacific salmon recovered from coastal southeastern Alaska identified by otolith thermal marks and coded wire tags. Fisheries Research 46:267-278.

Coutant, C.C. and C.H. Chen. 1993. Strontium microstructure in scales of freshwater and estuarine striped bass (Morone saxatilis) detected by laser ablation mass spectrometry. Can. J. Fish. Aquat. Sci. 50:1318-1323.

Cushing, D.H. and Burd, A.C. 1957. On herring of the southern North Sea. III. Fish. Invest. Ser. II, Vol. 10, 31 pp.

Czesny, S., K. Dabrowski, J.E. Christensen, J. Van Eenennaam, and S. Doroshov. 2000. Discrimination of wild and domestic origin of sturgeon ova based on lipids and fatty acid analysis. Aquaculture 189:145-153.

Das, U.N. 2001. Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? Prostaglandins Leukot Essent Fatty Acids.63:351-62.

Denoyer, E.R., K.J. Fredeen, and J.W. Hager. 1991. Laser solid sampling for inductively coupled plasma mass spectrometry. Anal. Chem. 63: 445A-457A.

Dickey-Collas, M. C.J.G. van Damme, L.A. Worsøe Clausen, and S.M.M. Fässler. 2005. Within Stock Structure and TACs: an investigation into the spawning origin of North Sea herring using otolith microstructure and the dynamics of Downs herring. Theme Session on Multidisciplinary Approaches to the Identification of Stock Structure of Small Pelagics: Implications for Assessment and Sustainable Management (K). ICES CM 2005/K:12. 14 pp.

Edmonds, J.S., M.J. Moran, N. Caputi, and M. Morita. 1989. Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in western Australian waters. Can. J. Fish. Aquat. Sci. 46: 50-54.

Edmonds, J.S., N. Caputi, and M. Morita. 1991. Stock discrimination by trace-element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water , marine teleost. Aust. J. Mar. and Freshwater Res. 42: 383-389.

Edmonds, J.S., R.C. Lenanton, N. Caputi, M. Morita. 1992. Trace elements in the otoliths of yellow-eye mullet (*Aldrichetta forsteri*) as an aid to stock identification. Fish. Res. (Amsterdam) 13: 39-51.

Elsdon, T.S. and B.M. Gillanders. 2006. Temporal variability in strontium, calcium, barium, and manganese in estuaries: Implications for reconstructing environmental histories of fish from chemicals in calcified structures. Estuarine, Coastal and Shelf Science 66: 147-156.

Fischer, J., R.L. Haedrich, and P.R. Sinclair. 1997. Interecosystem impacts of forage fish fisheries. Pages 311-321 in Forage fishes in marine ecosystems. Proceedings of the International Symposium on the role of forage fishes in marine ecosystems. Alaska Sea Grant College Program Report No. 97-01. University of Alaska Fairbanks, 1997.

FitzGerald, J.L., K.M. Baily, A.L. Brown, K.P. Severin. 2004. Elemental signatures in otoliths of larval walleye Pollock (Theragra chalcogramma) from the northeast Pacific Ocean. Fisheries Bulletin 102(4): 604-616.

Fraser, A.J., J.R. Sargent, J.C. Gamble, and D.D. Seaton. 1989. Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (Clupea harengus L.) larvae. Marine Chemistry 27:1-18.

Gao, Y.W., S.H. Joner, and G.G. Bargmann. 2001. Stable isotope composition of otoliths in identification of spawning stocks of Pacific herring (*Clupea pallasii*) in Puget Sound. Can. J. Fish. Aquat. Sci. 58:2113-2120.

Gaughan, D.J., K.V. White, and W.J. Fletcher. 2001. The links between functionally distinct assemblages of *Sardinops sagax*: larval advection across management boundaries. ICES Journal of Marine Science 58:597-606.

Grahl-Nielsen, O. and K.A. Ulvund. 1990. Distinguishing populations of herring by chemometry of fatty acids. American Fisheries Society Symposium 7:566-571.

Grahl-Nielsen, O. and O. Mjaavatten. 1992. Discrimination of striped bass stocks: A new method based on chemometry of the fatty acid profile in heart tissue. Trans. Amer. Fish. Soc. 121:307-314.

Grahl-Nielsen, O., M. Andersen, A.E. Derocher, C. Lydersen, Ø. Wiig, and K.M. Kovacs. 2004. Reply to comment on Grahl-Nielsen et al (2003): sampling, data treatment, and predictions in investigations on fatty acids in marine mammals. Marine Ecology Progress Series 281:303-306.

Grant W.S. and F.M. Utter. 1984. Biochemical population genetics of Pacific herring (*Clupea pallasii*). Canadian Journal of Fisheries and Aquatic Sciences 41: 856-864.

Gray, A.L. 1985. Solid sample introduction by laser ablation for inductively coupled plasma source mass spectrometry. Analyst (Cambridge, U.K.) 110: 551-556.

Grisdale-Helland, B., B. Ruyter, G. Rosenlund, A Obach, S.J. Helland, M.G. Sandberg, H. Standal and C. Rosjo. 2002. Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. Aquaculture 207:311-329.

Gunn, J.S., I.R. Harrowfield, C.H. Proctor, and R.E. Thresher. 1992. Electron microprobe microanalysis of fish otoliths – evaluation of techniques for studying age and stock discrimination. J. Exp. Mar. Biol. Ecol. 158: 1-36.

Gustafson, R.G. 2005. Multidisciplinary examination of Pacific herring (Clupea pallasii) population discreteness: the Cherry Point population and the USA's Endangered Species Act. Theme Session on Multidisciplinary Approaches to the Identification of Stock Structure of Small Pelagics: Implications for Assessment and Sustainable Management (K). ICES CM 2005/K:03. 23 pp.

Hagen, P., K. Munk, B. Van Alen, and B. White. 1995. Thermal mark technology for inseason fisheries management: A case study. Alaska Fisheries Research Bulletin 2(2):143-155.

Hamer, P.A. and G.P. Jenkins. 2007. Comparison of spatial variation in otolith chemistry for two fish species and relationships with water chemistry and otolith growth. Journal of Fish Biology 71 (4):1035-1055.

Hatch, G. 2004. Cell biology of cardiac mitochondrial phospholipids. Biochemistry and Cell Biology. 82:99112.

Hatfield, E.M.C., A.F. Zuur, J. Boyd, N. Campbell, J.C. Chubb, C.M. Collins, J. Coughlan, M.A. Cross, C.O. Cunningham, A.J. Geffen, K. MacKenzie, R.D.M. Nash, S. Jansen, S.M. Kay, C.J. Kelly, D.B. O'Leary, J. Schlickeisen, P.J. Schon, P.C. Watts, C. Zimmerman. 2005. WESTHER: A multidisciplinary approach to the identification of herring (*Clupea harengus L.*) stock components west of the British Isles using biological tags and genetic markers. Theme Session on Multidisciplinary Approaches to the Identification of Stock Structure of Small Pelagics: Implications for Assessment and Sustainable Management (K). ICES CM 2005/K:01. 18 pp.

Haugen, T., A. Kiessling, R.E. Olsen, M.B. Rørå, E. Slinde, and R. Nortvedt. 2006. Seasonal variations in muscle growth dynamics and selected quality attributes in Atlantic halibut (*Hippoglossus hippoglossus* L.) fed dietary lipids containing soybean and/or herring oil under different rearing regimes. Aquaculture 261:565-579.

Hauser, L. and R.D. Ward. 1998. Population identification in pelagic fish: The limits of molecular markers. Pages 191-224 in Advances in Molecular Ecology, G.R. Carvalho, Ed., IOS Press.

Hauser, L., G.R. Carvalho, and E.M.C. Hatfield. 2005. Big questions on small fish: Conceptual, methodological, and analytical challenges in the population identification of pelagic species. Paper presented at the Theme Session on Multidisciplinary Approaches to the Identification of Stock Structure of Small Pelagics: Implications for Assessment and Sustainable Management. ICES Council Meeting Documents: ICES CM 2005/K:06.

Hay, D. and P. McCarter. 1997. Larval distribution, abundance, and stock structure of British Columbia herring. Journal of Fish Biology 51(Suppl. A): 155-175.

Hay, D.E, P.B. McCarter, and K.S. Daniel. 2001. Tagging of Pacific herring *Clupea pallasii* from 1936-1992: a review with comments on homing, geographic fidelity and straying. Canadian Journal of Fisheries and Aquatic Sciences 58:1356-1370.

Hay, D.E. and S.M. McKinnell. 2002. Tagging along: association among individual Pacific herring (*Clupea pallasii*) revealed by tagging. Can. J. Fish. Aquat. Sci. 59:1960-1968.

Hazel, J.R. 1984. Effects of temperature on the structure and metabolism of cell membranes in fish. American Journal of Physiology 246:460-470.

Henderson, R.J., J.R. Sargent, and C.C.E. Hopkins. 1984. Changes in the content and fatty acid composition of lipid in an isolated population of capelin *Mallotus villosus* during sexual maturation and spawning. Marine Biology 78:255-263.

Henderson, R.J., and D.R. Tocher. 1987. The lipid composition and biochemistry of freshwater fish. Progress in lipid research: 281-347.

Hilborn, R., T.P. Quinn, D.E. Schindler, and D.E. Rogers. 2003. Biocomplexity and fisheries sustainability. Proceedings of the National Academy of Sciences 100(11):6564-6568.

Hourston, A.S. 1982. Homing by Canada's west coast herring to management units and divisions as indicated by tag recoveries. Can. J. Fish. Aquatic Sci 39(10): 1414-1422.

Huberty, C. J. 1994. *Applied Discriminant Analysis*. Wiley Series in Probability and Mathematical Statistics. John Wiley and Sons, Inc. New York.

Huse, G., S. Railsback, and A. Ferno. 2002. Modeling changes in migration pattern of herring: collective behavior and numerical domination. Journal of Fish Biology 60:571-582.

Huynh, M.D., D.D. Kitts, C. Hu, and A.W. Trites. 2007. Comparison of fatty acid profiles of spawning and non-spawning Pacific herring, *Clupea harengus pallasii*. Comparative Biochemistry and Physiology, Part B 146:504-511.

Iverson, S.J., K.J. Frost, and L.F. Lowry. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Mar. Ecol. Prog. Ser. 151: 255-271.

Jobling, M., A.V. Larsen, B. Andreassen, T. Sigholt, and R.L. Olsen. 2002. Influence of dietary shift on temporal changes in fat deposition and fatty acid composition of Atlantic salmon post-smolt during the early phase of seawater rearing. Aquaculture Research 33:875-889.

Joensen, H. and O. Grahl-Nielsen. 2000. Discrimination of *Sebastes viviparus, Sebastes marinus*, and *Sebastes mentella* from Faroe Islands by chemometry of the fatty acid profile in the heart and gill tissues and in the skull oil. Comparative Biochemistry and Physiology, Part B 126:69-79.

Joensen, H., P. Steingrund, I. Fjallstein, and O. Grahl-Nielsen. 2000. Discrimination between two reared stocks of cod (*Gadus morhua*) from the Faroe Islands by chemometry of the fatty acid composition in the heart tissue. Marine Biology 136: 573-580.

Jonsdottir, I.G., S.E. Campana, and G. Marteinsdottir. 2006. Stock structure of Icelandic cod *Gadus morhua L*. based on otolith chemistry. Journal of Fish Biology 69 (Supplement C):136-150.

Joyce, T.L., D.G. Evans, and R. Riffe. 1996. Otolith marking of pink salmon in Prince William Sound hatcheries, 1995, Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 95320C), Alaska Department of Fish and Game, Commercial Fisheries Management and Development Division, Cordova, AK.

Kalish, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132: 151-178.

Kalish, J.M. 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fish. Bull. 88: 657-666.

Kalish, J. M. 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus.* Marine Ecology Progress Series, 74:137–159.

Kennedy, B. P., Klaue, A., Blum, J.D., Folt, C.L., and Nislow, K.H. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Can J. Fish. Aqua. Sci., 59: 925–929.

Kiessling, A., J. Pickova, L. Johansson, T. Åsgård, T. Storebakken, K-H. Kiessling. 2001. Changes in fatty acid composition in muscle and adipose tissue of farmed rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age. Food Chemistry 73:271-284.

Kirsch, P.E., S.J. Iverson, W.D. Bowen, S.R. Kerr, and R.G. Ackman. 1998. Dietary effects on the fatty acid signature of whole Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 55:1378-1386.

Kwetegyeka, J., G. Mpango, and O. Grahl-Nielsen. 2006. Fatty acid composition of muscle and heart tissue of Nile perch, Lates niloticus, and Nile tilapia, Oreochromis niloticus, from various populations in Lake Victoria and Kioga, Uganda. African Journal of Aquatic Sciences 31(2):297-304.

Letcher, B.H., and T.L. King. 1999. Targeted stock identification using multilocus genotype 'familyprinting'. Fisheries Research 43:99-111.

Logerwell, E.A., and N.B. Hargreaves. 1997. Seabird impacts on forage fish: Population and behavioral interactions. Pages 191-195 In: Forage fishes in marine ecosystems. Proceedings of the International Symposium on the role of forage fishes in marine ecosystems. Alaska Sea Grant College Program Report No. 97-01. University of Alaska Fairbanks, 1997.

Ludsin, S.A., B.A. Fryer, and J.E. Gagnon. 2006. Comparison of solution-based versus laser ablation-inductively coupled plasma mass spectrometry for analysis of larval fish otolith microchemical composition. Transactions of the American Fisheries Society 135:218-231.

MacDonald, J.I., J.M.G. Shelley, and D.A. Crook. 2008. A method for improving the estimation of natal chemical signatures in otoliths. Transactions of the American Fisheries Society 137: 1674-1682.

MacKenzie, K., and P. Abaunza. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. Fisheries Research 38:45-56.

McGarigal, K., S. Cushman and S. Stafford. 2000. Multivariate statistics for wildlife and ecology research. Springer-Verlag. New York. 283 p.

McKenzie, D.J., G. Piraccini, C. Agnisola, J.F. Steffensen, P. Bronzi, C.L. Bolis, B. Tota, and E.W. Taylor. 1999. The influence of dietary fatty acid composition on the respiratory and cardiovascular physiology of Adriatic sturgeon (Acipenser naccarii): a review. Journal of Applied Ichthyology 15: 265-269.

McKenzie, D.J. 2001. Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. Comp. Bio. Physio. Pt A. 128:607-627.

McPherson, A.A., P.T. O'Reilly, and C.T. Taggart. 2004. Genetic differentiation, temporal stability, and the absence of isolation by distance among Atlantic herring populations. Transactions of the American Fisheries Society 133:434-446.

McQuinn, I.H. 1997. Metapopulations and the Atlantic herring. Reviews in Fish Biology and Fisheries 7(3):297-329.

Metcalfe, N.B., Wright, P.J., and Thorpe, J.E. 1992. Relationships between social status, otolith size at first feeding and subsequent growth in Atlantic salmon (*Salmo salar*). Journal of Animal Ecology, 61:585-589.

Miller, J.A., M.A. Banks, D. Gomez-Uchida, and A.L. Shanks. 2005. A comparison of population structure in black rockfish (*Sebastes melanops*) as determined with otolith chemistry and microsatellite DNA. Canadian Journal of Fisheries and Aquatic Science 62:2189-2198.

Milton, D.A. and S.R. Chenery. 1998. The effect of otolith storage methods on the concentrations of elements detected by laser-ablation ICPMS. Journal of Fish Biology 53:785-794.

Mitchell, D.M. 2006. Biocomplexity and metapopulation dynamics of Pacific herring (*Clupea pallasii*) in Puget Sound, Washington. M.S. Thesis. University of Washington. 75 pp.

Molony, B.W. 1996. Episodes of starvation are recorded in the otoliths of juvenile *Ambassis vachelli* (Chandidae), a tropical estuarine fish. Marine Biology. 125 439-446.

Munro, A.R., B.M. Gillanders, T.S. Elsdon, D.A. Crook, and A.C. Sanger. 2008. Enriched stable isotope marking of juvenile golden perch (Macquaria ambiqua) otoliths. Canadian Journal of Fisheries and Aquatic Sciences 65:276-285.

O'Connell, M., M.C. Dillon, J.M. Wright, P. Bentzen, S. Merkouris, and J. Seeb. 1998. Genetic structuring among Alaskan Pacific herring populations identified using microsatellite variation. Journal of Fish Biology 53:150-163.

Oliva, M.E., and I. Ballón. 2002. Metazoan parasites of the Chilean hake *Merluccius gayi gayi* as a tool for stock discrimination. Fisheries Research 56:313-320.

Otis, E.O., W.R. Bechtol, and W.A. Bucher. 1998. Coping with a challenging stock assessment situation: the Kamishak Bay sac-roe herring fishery. Pages 557-573 In Fishery Stock Assessment Models: Proceedings of the International Symposium on Fishery Stock Assessment Models for the 21st Century, October 8-11, 1997, Anchorage, Alaska. Eds. F. Funk, T.J. Quinn, J. Heifetz, J.N. Ianelli, J.E. Powers, J.F. Schweigert, P.J. Sullivan, and C.-I. Zhang. University of Alaska Sea Grant College Program AK-SG-98-01

Otis, E.O., and R. Heintz. 2003. Evaluation of two methods to discriminate Pacific herring (*Clupea pallasii*) stocks along the northern Gulf of Alaska. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 02538), Alaska Department of Fish and Game, Division of Commercial Fisheries, Homer, Alaska. 37 pp.

Overholtz, W.J. 2002. The Gulf of Maine-Georges Bank Atlantic herring (*Clupea harengus*): spatial pattern analysis of the collapse and recovery of a large marine fish complex. Fisheries Research 57:237-254.

Owen, A.J., B. A. Peter-Przborowska, A. J. Hoy and P. L. McLennan. 2004. Dietary fish oil dose- and time-response effects on cardiac phospholipid fatty acid composition. Lipids 39:955961.

Pannella, C. 1980. Growth patterns in fish sagittae. In: Rhoads, D.C., and R.A. Lutz (eds.), Skeletal growth of aquatic organisms--Biological records, and environmental change, p. 519-557. Plenum Press, NY.

Patterson, H.M., M.J. Kingsford, M.T. McCulloch. 2004. Elemental signatures of Pomacentrus coelestis at multiple spatial scales on the Great Barrier Reef, Australia. Marine Ecology Progress Series 270:229-239.

Pawson, M.G., and S. Jennings. 1996. A critique of methods for stock identification in marine capture fisheries. Fisheries Research 25:203-217.

Peng, J., Y. Larondelle, D. Pham, R.G. Ackman, and X. Rollin. 2003. Polyunsaturated fatty acid profiles of whole body phospholipids and triacylglycerols in anadromous and landlocked Atlantic salmon (*Salmo salar* L.) fry. Comparative Biochemistry and Physiology Part B 134:335-348.

Pickova, J., P.C. Dutta, P.O. Larsson, and A. Kiessling. 1997. Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (*Gadus morhua*) stocks. Can. J. Fish. Aquat. Sci. 54:2410-2416.

Radtke, R.L. and D.J. Shafer. 1992. Environmental sensitivity of fish otolith microchemistry. Aust. J . Mar. Freshwater Res. 43: 935-951.

Rogers, D.E., K.N. Schnepf, and P.R. Russell. 1984. Feasibility of using scale analysis methods to identify Bering Sea herring stocks. Annual Report to National Marine Fisheries, Contract Number 83-ABC-00165. Fisheries Research Institute, School of Fisheries, University of Washington, Seattle, WA. 49 pp.

Rollin, X., J. Peng, D. Pham, R. Ackman, and Y. Larondelle. 2003. The effects of dietary lipid and strain difference on polyunsaturated fatty acid composition and conversion in anadromous and landlocked salmon. Comparative Biochemistry and Physiology Part B 134:349-366.

Rottiers, D.V. 1993. Elemental composition of a migratory and a land-locked strain of Atlantic salmon *Salmo salar*. Comparative Biochemistry and Physiology, A 104(1):93-100.

Rowell, K. A. 1981. Separation of spawning stocks of Bering Sea herring based on scale growth patterns. In B.R. Melteff and V.G. Westpestad (ed.) Proceedings Alaska herring symposium. Alaska Sea Grant Report No. 80-4. Fairbanks, Alaska.

Ruttenberg, B.I., S.L. Hamilton, M.J.H. Hickford, G.L. Paradis, M.S. Sheehy, J.D. Standish, O. Ben-Tzvi, and R.R. Warner. 2005. Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. Marine Ecology Progress Series 297: 273-281.

Ruzzante, D.E., S. Mariani, D. Bekkevold, C. André, H. Mosegaard, L.A.W. Clausen, T.G. Dahlgren, W.F. Hutchinson, E.M.C. Hatfield, E. Torstensen, J. Brigham, E.J. Simmonds, L. Laikre, L.C. Larsson, R.J.M. Stet, N. Ryman, and G.R. Carvalho. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Proceedings of the Royal Society B 273:1459-1464.

Sadovy, Y., and K.P. Severin. 1994. Elemental patterns in red hind (Epinephelus guttatus) otoliths from Bermuda and Puerto Rico reflect growth rate, not temperature. Canadian Journal of Fisheries and Aquatic Sciences 51:133-141.

Schweigert, J.F., and R.E. Withler. 1990. Genetic differentiation of Pacific herring based on enzyme electrophoresis and mitochondrial DNA analysis. In N.C. Parker, A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, and G.A. Winans (eds.) Fish Marking Techniques, American Fisheries Society Symposium 7. 879 pp.

Schweigert, J.F. 1990. Comparison of morphometric and meristic data against truss networks for describing Pacific herring stocks. In N.C. Parker, A.E. Giorgi, R.C. Heidinger, D.B.

Jester, Jr., E.D. Prince, and G.A. Winans (eds.) Fish Marking Techniques, American Fisheries Society Symposium 7. 879 pp.

Secor, D.H. 1992. Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay striped bass *Morone saxatilis*. Fish. Bull. 90: 798-806.

Secor, D.H. 2007. The year-class phenomenon and the storage effect in marine fishes. Journal of Sea Research 57:91-103.

Severin, K.P., J. Carroll, and B.L. Norcross. 1995. Electron microprobe analysis of juvenile walleye pollock, Theragra chalcogramma, otoliths from Alaska: a pilot stock separation study. Environmental Biology of Fishes 43: 269-283.

Sinclair, D.J. 2005. Correlated trace element "vital effects" in tropical corals: A new geochemical tool for probing biomineralization. Geochimica et Cosmochimica Acta 69(13):3265-3284.

Small, M., J.L. Loxteman, A.E. Frye, J.F. Von Bargen, C. Bowman, and S.F. Young. 2005. Temporal and spatial genetic structure among some Pacific herring populations in Puget Sound and the Southern Strait of Georgia. Transactions of the American Fisheries Society 134 (5):1329-1341.

Smith, M. K. 1992. Regional differences in otolith morphology of the deep slope red snapper *Etelis carbunculus*. Canadian Journal of Fisheries and Aquatic Sciences 49:795–804.

Smith, P.J., and A. Jamieson. 1986. Stock discreteness in herrings: A conceptual revolution. Fisheries Research 4:223-234.

Stearns, S.C. 1992. The evolution of life histories. Oxford University Press, New York. 264 pp.

Stenslokken K.O., L. Sundin, and G.E. Nilsson. 2002. Cardiovascular effects of prostaglandin F(2 alpha) and prostaglandin E(2) in Atlantic cod (*Gadus morhua*). J Comp Physiol [B] 172:363-369.

Stephenson, R.L. 1999. Stock complexity in fisheries management: a perspective of emerging issues related to populations subunits. Fisheries Research 43:247-249.

Swain, D.P., and C.J. Foote. 1999. Stocks and chameleons: the use of phenotypic variation in stock identification. Fisheries Research 43:113-128.

Swan, S.C., A.J. Geffen, J.D.M. Gordon, B. Morales-Nin, and T. Shimmield. 2006. Effects of handling and storage methods on the concentrations of elements in deep-water fish otoliths. Journal of Fish Biology 68:891-904.

Swearer, S.E., G.E. Forrester, M.A. Steele, A.J. Brooks, and D.W. Lea. 2003. Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblage. Estuarine Coastal and Shelf Science 56: 1111-1123.

Tanasichuk, R.W., D.M. Ware, W. Shaw, and G.A. McFarlane. 1991. Variations in diet, daily rations, and feeding periodicity of Pacific hake (*Merluccius productus*), and spiny dogfish (*Squalus acanthias*) off the lower west coast of Vancouver Island. Can. J. Fish. Aquat. Sci. 48: 2118-2128.

Tester, A.L. 1949. Populations of herring (*Clupea pallasii*) in the coastal waters of British Columbia. J. Fish. Res. Board Can. 7:403-420.

Thiemann, G.W., S.M. Budge, W. D. Bowen, and S.J. Iverson. 2004. Comment on Grahl-Nielsen et al. (2003) 'Fatty acid composition of the adipose tissue of polar bears and of their prey: ringed seals, bearded seals and harp seals'. Marine Ecology Progress Series 281:297-301.

Thomassen, M., and C. Røsjø. 1989. Different fats in feed for salmon: influence on sensory parameters, growth rate and fatty acids in muscle and heart. Aquaculture 79:129-135.

Thorrold, S.R., C. Latkoczy, R.K. Swart, and C.M. Jones. 2001. Natal homing in a marine fish metapopulation. Science 291:297-299.

Thorrold, S.R., G.P. Jones, S. Planes, and J.A. Hare. 2006. Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 63:1193-1197.

Thresher, R.E., C.H. Proctor, J.S. Gunn, and I.R. Harrowfield. 1994. An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperature groundfish, *Nemadactylus macropterus*. Fish. Bull: 92: 817-840.

Thresher, R.E. 1999. Elemental composition of otoliths as a stock delineator in fishes. Fisheries Research 43:165-204.

Turner, J.P., and J.R. Rooker. 2005. Effect of diet on fatty acid compositions in *Sciaenops ocellatus*. Journal of Fish Biology 67:1119-1138.

Viga, A., and O. Grahl-Nielsen. 1990. Genotypic and phenotypic fatty acid composition in the tissues of salmon, *Salmo salar*. Comp. Biochem. Physiol. 96B: 721-727.

Volk, E.C., A. Blakely, S.L. Schroder, and S.M. Kuehner. 2000. Otolith chemistry reflects migratory characteristics of Pacific salmonids: using otolith core chemistry to distinguish maternal associations with sea and freshwaters. Fisheries Research 46:251-266.

Waldman, J.R. 1999. The importance of comparative studies in stock analysis. Fisheries Research 43: 237-246.

Walker, R.V. and K.N. Schnepf. 1982. Scale pattern analysis to estimate the origin of herring in the Dutch Harbor fishery. Final Report to the Alaska Department of Fish and Game, FRI-UW-8219. Fisheries Research Institute, School of Fisheries, University of Washington, Seattle, WA. 21 pp.

Walton, M.J., R.J. Henderson, and P.P. Pomeroy. 2000. Use of blubber fatty acid profiles to distinguish dietary differences between grey seals from two UK breeding colonies. Marine Ecology Progress Series 193:201-208.

Walton, M. and P. Pomeroy. 2003. Use of blubber fatty acid profiles to detect inter-annual variations in the diet of grey seals *Halichoerus grypus*. Marine Ecology Progress Series 248:257-266.

Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. Journal of Heredity 89:438-450.

Waples, R.S. and O. Gaggiotti. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology 15:1419-1439.

Ware, D.M. and R.W. Tanasichuk. 1989. Biological basis of maturation and spawning waves of Pacific herring (Clupea harengus pallasi). Canadian Journal of Fisheries and Aquatic Sciences 46(10):1776-1784.

Ware, D.M, C. Tovey, D. Hay, and B. McCarter. 2000. Straying rates and stock structure of British Columbia herring. Science Advisory Secretariat Research Document 2000/006. 32 pp.

Ware, D.M. and C. Tovey. 2004. Pacific herring disappearance and recolonization events. Canadian Science Advisory Secretariat Research Document 2004/008. 52 pp.

Wells, B.K., Friedland, K.D., and Clarke, L.M. 2003. Increment patterns in otoliths and scales from mature Atlantic salmon *Salmo salar*. Marine Ecology Progress Series, 262: 293-298.

Westpestad, V.G. and E. Moksness. 1989. Observations on growth and survival during the early life history of Pacific herring *Clupea pallasii* from Bristol Bay, Alaska in a marine mesocosm. Fishery Bulletin 88(1):191-200.

Wheeler, J.P. and G.H. Winters. 1984. Homing of Atlantic herring (*Clupea harengus harengus*) in Newfoundland waters as indicated by tagging data. Can. J. Fish. Aquatic Sci. 41(1): 108-117.

Zhang, Z., and Runham, N. W. 1992. Otolith microstructure pattern in *Oreochromis niloticus*. Jour. Fish Biol. 40:325–332.

Zimmerman, C.E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. Canadian Journal of Fisheries and Aquatic Sciences 62: 88-97.

Other References

C. Burkey, Alaska Department of Fish and Game, personal communication, 29 March 2001.

T. Baker, Alaska Department of Fish and Game, personal communication, 27 June 2008.

H. Woody, Sitka Tribe of Alaska, personal communication, 30 April, 2009.

S. Trumble, Baylor University, personal communication, 14 April 2009.

R. Brown, US Fish and Wildlife Service-Fairbanks, personal communication, 29 May 2009.