Garth Griffin, Branch Chief
Protected Resources Division
National Marine Fisheries Service
U.S. Department of Commerce
525 NE Oregon Street
Portland, Oregon
97232-2737

May 14, 2004

RE: Significant New Information on Cherry Point Herring

Dear Mr. Griffin;

I write on behalf of Northwest Ecosystem Alliance, Center for Biological Diversity, Ocean Advocates, People for Puget Sound, Public Employees for Environmental Responsibility, Friends of Puget Sound, and Sam Wright to submit significant new information for your consideration regarding Endangered Species Act listing of the Cherry Point Distinct Population Segment of Pacific herring.

On January 22, 2004, NMFS received a formal and detailed petition to list the Cherry Point Distinct Population Segment of Pacific herring under the Endangered Species Act. The petition presented substantial new information that Cherry Point herring are discrete from other herring populations and significant to the taxon.

Since that date, additional studies have been completed or have come to our attention that provide additional significant new information that Cherry Point herring are a distinct population segment facing imminent threat of extinction. First, the Washington State Department of Fish and Wildlife has completed an analysis of microsatellite DNA that shows that the Cherry Point herring are genetically similar to each other, and genetically distinct from other herring stocks in the Puget Sound and Strait of Georgia. The analysis is attached as a MS Word file titled "WDFW Herring Genetics Report 4.27.04.doc" and the document is: Small, M.P., J. Loxterman, and S. Young. 2004. A microsatellite DNA investigation of Pacific herring (*Clupea pallasi*) population structure in Puget Sound, Washington. Washington State Department of Fish and Wildlife. Olympia, WA.

Secondly, the 2004 Cherry Point herring spawn appears to be dismal. To date, only 350 tons of spawning Cherry Point herring has been recorded, compared to as much as 1200 tons by this time during the spawn over the last five years. There is reportedly only a low number of herring holding offshore, and those attempting to spawn have been subject to intense predation by birds. For information and updates on the status of the 2004 Cherry Point herring spawn, we request that you contact the Washington State Department of Fish and Wildlife at 360-902-2200.

Third, research published in September 2003 regarding herring behavior and the effects of noise pollution suggests that Cherry Point herring communication may be harmfully impacted by industrial noise associated with the operation of docks, vessels, and other machinery within and around their spawning grounds. The publication is attached as a PDF file titled "Herring communication 9.15.03.pdf" and the document is: Wilson, B., R.S. Batty, and L. M. Dill. 2003. Pacific and Atlantic herring produce burst pulse sounds. Proceedings of the Royal Society. London.

Finally, we submit a study showing that loss of species' populations are a prelude to that species extinction, and that, conversely, maintaining population diversity is key to species conservation. The publication is attached as a PDF file titled "Conserving Populations.pdf" and the document is: G. Ceballos and P.R. Ehrlich. 2002. Mammal population losses and the extinction crisis. Science. Vol. 296:904-907.

Thank you for reviewing this information and considering it as part of the substantial information already presented which demonstrates that listing of the Cherry Point Distinct Population Segment of Pacific herring may be warranted. For your convenience, this letter is included as a MS Word doc titled "CHP new info letter 5.14.04.doc."

Sincerely,

/s/
Dave Werntz
Northwest Ecosystem Alliance
1208 Bay Street, Ste. 201
Bellingham, WA 98225
360-671-9950 ex. 14

# A microsatellite DNA investigation of Pacific herring (*Clupea pallasi*) population structure in Puget Sound, Washington

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Washington State Department of Fish and Wildlife

April 27, 2004

Abstract:
Herring collections from Washington State, British Columbia, Alaska and California were genetically characterized using 12 microsatellite loci. Loci were highly variable with up to 70 alleles per locus (mean = 30.67 alleles) and observed heterozygosity was high (mean = 0.823). Molecular variance indicated significant structure within the entire data set and within Puget Sound collections, in both cases with over twice as much variance among sites as among year classes, although both were significant. Pairwise genotypic tests showed significant differences in genotypic distributions among most sites and few significant differences among year classes within a site. Within Puget Sound, Cherry Point herring collections had no differences in genotypic distributions among year classes and in multidimensional scaling and principle component analyses Cherry Point collections formed a distinctive group. Cherry Point herring have a late spawning time in comparison to other Puget Sound herring. Results suggest run-timing differences and geography interact to generate genetic structure in Pacific herring.

Introduction:
Herring (*Clupea pallasi*) are small marine fish that aggregate in large schools. Since herring serve as a prey base for other marine fish, birds and mammals, they are critical components in oceanic and near-shore food chains. Herring are also important as bait for recreational fisheries and as commercial fisheries in Washington State, British Columbia (BC), Alaska (AK) and other Pacific Rim regions. Starting near the end of January to as late as June in northern areas, herring school in aggregates of millions of fish near inshore habitat in preparation to spawn a few weeks later. Herring broadcast their spawn, releasing adhesive eggs that settle upon eelgrass, algae and solid substrate in lower intertidal and upper subtidal shoreline areas. Spawning sites and associated aggregations have patchy distributions since they are separated by unsuitable habitat. According to tagging studies in BC, herring return to spawning sites with levels of fidelity varying from 64% to 96% (Ware *et al.* 2000, Ware and Schweigert 2001, 2002). Fidelity (and straying) appears to be influenced by the biomass of the spawning stock – the larger the stock, the higher the exportation of individuals (Ware and Schweigert 2001). The genetic structure of herring in BC reflects this straying rate in high connectivity among BC herring spawning sites (Beacham *et al.* 2002), with higher connectivity among sites than between year classes within sites. Prior to this study, we were unable to compare site-to-site variation within years and year-to-year variation within sites in Puget Sound.

Herring in Puget Sound spawn in well-defined locations and at specific times (Stout *et al.* 2001). Differences in spawn timing could act as a reproductive barrier allowing genetic differentiation of stocks. In particular, Cherry Point with its late spawning time from April through early June (Stout *et al.* 2001) may be differentiated from other groups spawning from late January to early April (http://wdfw.wa.gov/fish/forage). In this study we examine regional and temporal population structure of herring in Puget Sound and compare them to herring from Alaska, Strait of Georgia (BC) and California.

Materials and methods: Genotypes for 1511 herring from Puget Sound, Strait of Georgia, Alaska and California (Table 1, Figure 1) were assessed at 12 microsatellite DNA loci

(Table 2). Table 1 shows the total number of samples collected and the number of samples included per collection: samples were included in the analysis if they amplified at five or more loci (N = 1453). Laboratory conditions and primer sequences for loci are outlined in Table 3. Samples were run on an ABI 3730 automated sequencer. Collections were tested for deviations from Hardy Weinberg equilibrium (HWE) at each locus and over all loci using GENEPOP 3.3 (Raymond and Rousset 1995). Other statistics included genotypic disequilibrium (are different loci associated with each other through physical linkage on the same chromosome or through non-random mating?), gene diversity (a measure of expected heterozygosity corrected for sample size), allelic richness (number of alleles per collection corrected for sample size), were assessed using GENEPOP 3.3, FSTAT2.9.3 (Goudet 2001) and MSA (Dieringer and Schlötterer 2002). Collections were tested for differences in genotypic distributions with pairwise Chi-square tests using GENEPOP 3.3 with 200 batches and 2000 iterations. All test results were adjusted for multiple comparisons using Bonferroni corrections. Genetic distances (Nei's chord distance, Nei *et al.*1983) among collections were estimated using MSA. Distances among collections were plotted in a multidimensional scaling (MDS) analysis using NTSYS-pc (Rohlf 1993) to visualize possible relationships among collections. Distances were also plotted in a consensus neighbor-joining (NJ) tree using PHYLIP (Felsenstein 1993) employing a bootstrapping protocol to estimate the reliability of branching in the NJ tree. FSTAT was used to calculate $\theta$, an estimate of $F_{ST}$ (a measure of genetic structure indicating differentiation among collections) and the 95% confidence interval surrounding the value. Underlying trends in the data set were examined in a principle components analysis with 10,000 randomizations using PCAGEN 1.2.1 (Goudet 1999). Partitioning of molecular variance was examined with an analysis of molecular variance (AMOVA, Excoffier *et al*. 1992) using ARLEQUIN 2.001 (Schneider *et al.* 2000) using 10,000 randomizations. Collections were tested as a group then separated by location and then separated by year class to test how much molecular variance was partitioned by location and by year class.

Results: Individuals included in the study amplified at five or more loci (Table 1). The 02Port Gamble collection had the least successful amplifications, with 62 individuals included in this study out of the 96 individuals collected for the study. 02Port Gamble had a low of 22 individuals amplifying at *Cha-27* and a high of 62 individuals amplifying at *Cha-113* and at *Cha-107*, for an average of 45 successful amplifications per locus: most individuals were characterized by genotypes at a subset of the loci and only 10 were characterized by the full suite. Deviations from Hardy-Weinberg equilibrium (HWE) for homozygote excess were detected in 23 tests of loci within collections (underlined in Table 2), but none of these deviations remained significant after Bonferroni corrections. When calculated over all loci, four collections deviated from HWE with homozygote excesses (bold in Table 1). The Alaska collections, 99Port Gamble, and 99Cherry Point (non-spawners) had significant, positive $F_{IS}$ value, indicating that collections were admixtures of populations, or had experienced inbreeding, or had null alleles. When tested over all collections, six loci deviated from HWE before Bonferroni corrections (Table 3, underlined values), with four of these deviations remaining significant after Bonferroni corrections (values in bold type). Since *Cha-8* was out of HWE in most collections (before corrections) and out of HWE over all collections, we removed this

locus from the rest of the analyses. In genotypic disequilibrium tests over all collections, six locus pairs were linked statistically by virtue of strong linkage within a single collection (data not shown). In tests within collections, 13 locus pairs were linked (Table 2) with *Cpa-A* and *Cpa-172* linked in two collections, 98San Francisco and 00Cherry Point. Other locus pairs were linked in one collection. Since most locus pairs were linked in a single collection, linkage was more likely due to admixture of populations within collections or possibly due to non-random mating through small numbers of effective breeders rather than physical linkage on the same chromosome. Positive $F_{IS}$ values in most collections also support hypotheses of population admixture or possibly inbreeding. However, given the immense breeding aggregate sizes, inbreeding is unlikely. Gene diversity (expected heterozygosity) was comparable in all collections. Allelic richness was highest in 96Norton Sound and lowest in 02Port Gamble.

Most pairwise tests indicated significant differences in genotype distributions among collections from different sites and overlap between collections from different years at the same sites: Cherry Point, Squaxin Pass, Port Gamble (Table 4, lower matrix). 99Cherry Point non-spawners were undifferentiated from Cherry Point spawners and several other Puget Sound collections (including Northumberland, BC) and had a significant, positive $F_{IS}$ value, suggesting that Cherry Point non-spawners included herring from different breeding aggregates. We detected no difference between herring collections from AK (Table 4) even though collection sites were at least 3000 km apart. Herring from these two regions were differentiated in previous studies using microsatellites and other markers (Bentzen *et al.* 1998 and references within, O'Connell *et al.* 1998). Significant overlap was also found between Port Gamble and AK collections, between Semiahmoo and Port Gamble collections, between 99Fidal and 02Semiahmoo, and between 99Semiahmoo and 99Northumberland, BC (Table 4). Results suggest reproductive isolation of the Cherry Point spawning group and some associations among other Puget Sound spawning groups.

The multidimensional scaling (MDS) plot suggested groups based upon region (Figure 2). 98San Francisco separated from the rest of the collections along the first axis: distances involving 98San Francisco were so large that they compressed the rest of the collections onto one side of the first axis. Genetic distances were thus plotted without 98San Francisco in a second MDS plot (Figure 3). Port Gamble and AK collections separated somewhat from Puget Sound collections along the first and second axes. Gamble Cherry Point spawner collections separated from other collections along the third axis. Cherry Point non-spawners occupied a place in the center of the plot. The stress values in both MDS plots (0.2 = fair and 0.4 = poor; NTSYS-pc documentation) indicate that reducing relationships between collections to three dimensions distorted some of those relationships.

In the consensus neighbor-joining tree (Figure 4) based upon Nei's genetic chord distances, the Cherry Point spawner collections were on a single branch with 65 % bootstrap support. The AK collections formed a branch with 97% bootstrap support and Squaxin Pass collections formed a branch with 77% bootstrap support.

The overall θ value from FSTAT, 0.006, was significantly different from 0 with a 95% confidence interval of 0.003 to 0.01 when bootstapped over all loci and a standard error of 0.002 when jackknifed over all loci, indicating low but significant genetic structure in the data set.

In the principle components analysis, global $F_{ST}$ was 0.0107 ($P$ = 0.001), indicating significant genetic structure in the data set. Similar to the MDS, the San Francisco collection compressed other collections to one side (data not shown) and the analysis was conducted without San Francisco (Figure 5). Global $F_{ST}$ without San Francisco was 0.0095 ($P$ = 0.0001) and $F_{ST}$ values for the first three axes were 0.00215, 0.00134, and 0.00114, and percent inertia for each axis was 22.6, 14.11 and 11.96 respectively ($P$ < 0.001 for each axis for the proportion of inertia of each axis). The first and third axes were plotted since this displayed better distinction among sites. Results were similar to the MDS without San Francisco: AK collections separated from the Puget Sound collections along the first axis with Port Gamble and Northumberland collections on the same side of axis 1. Cherry Point spawners formed a cluster and 99Cherry Point non-spawners and 99 Fidalgo plotted near the center of all three axes.

In the AMOVA analysis (Table 5), the overall $F_{ST}$ value over all loci was low but significant. When collections were divided by site (Cherry Point, Semiahmoo, Squaxin Pass, Fidalgo Bay, Port Gamble, Northumberland, San Francisco and AK), the $F_{ST}$ value was similar (see "within collections" under "Divided by site", Table 5) since the majority of the variance was within collections. Although there was significant variance among year-classes within sites, there was over twice as much variance among sites than among year classes (see "among sites" under "Divided by site" and "among years" under "Divided by year", Table 5). When the Puget Sound collections were analyzed separately (with Northumberland, BC from Strait of Georgia included), the $F_{ST}$ value was lower but still significant. However, the variance among sites was an order of magnitude greater than variance among years (see "among sites" and "among years" under "Divided by site" and "Divided by year" in Table 5), supporting a hypothesis that herring populations in Puget Sound are partitioned into breeding groups according to spawning site.

Discussion:

The most interesting result from this study is the distinction of the Cherry Point spawning aggregation. Cherry Point collections were similar to each other and different from Semiahmoo herring, a few miles distant, and from other spawning groups in Puget Sound and Strait of Georgia. This is remarkable, given that in BC herring Ware and Schweigert (2001) report stray rates up to 36%, suggesting high connectivity among herring stocks within and between regions (Beacham *et al.* 2002). However, Cherry Point herring are distinguished from other Puget Sound herring by their April spawning time, generally weeks after other groups have spawned in Puget Sound and the Strait of Georgia. This late spawning acts as a temporal barrier that maintains a degree of reproductive isolation between Cherry Point and other Puget Sound and Strait of Georgia groups. Beacham *et al.* (2002) also found that Cherry Point herring were differentiated from Strait of Georgia stocks.

Although collected on the same day as the 99Cherry Point spawner group, the 99Cherry Point non-spawners were in pre-spawning condition (Paul Bentzen, personal communication). The non-spawners appeared to be composed of individuals from Cherry Point and possibly other spawning groups since genotypic distributions overlapped with Semiahmoo, Port Gamble and Northumberland.

The most puzzling result from this study is our failure to detect differentiation between herring from the Bering Sea and Prince William Sound in Alaska. Allozyme analysis (Grant and Utter 1984), microsatellites (O'Connell *et al.* 1998) and mitochondrial analysis (Bentzen *et al.* 1998) report significant genetic differences between herring collected from these locations. By increasing the number of microsatellite loci, we expected to increase the resolution of their genetic relationship. The high stray rate reported for BC herring (Ware and Schweigert 2001) is unlikely between Norton Sound and Prince William Sound, a minimum distance of around 3000 kilometers. Ware *et al.* (2000) found that of the herring that strayed, less than 8% dispersed 800 or more kilometers. This lack of observed differentiation between spawners from Norton Sound and Prince William Sound may indicate that the populations are so large that same-sized alleles arise in the populations by different evolutionary pathways, making the collections appear undifferentiated. The extreme haplotype diversity in these herring (Paul Bentzen, preliminary summary to WDFW, March 13, 2004) would support a hypothesis of large effective population sizes. Alternatively, or in addition, interannual variability may be so high in AK herring stocks that at times they are genetically undifferentiated simply by chance. In another puzzling comparison, the AK stocks were undifferentiated from Port Gamble and Northumberland herring. This may have been a result of poor genetic characterization of the 02Port Gamble stock, but this fails to account for relationships with 99Port Gamble and 99Northumberland. Pairwise genotypic tests are highly sensitive and detect even subtle differences among subpopulations (Balloux and Lugon-Moulin 2002). Thus, the lack of differentiation is puzzling.

Straying may occur among some Puget Sound and Strait of Georgia stocks similar to straying among BC stocks. Using microsatellite analysis, Beacham *et al.* (2002) found little differentiation among British Columbia stocks within and between regions, with the exception of stocks with different spawn timing. In BC herring, annual variation was greater than differences between stocks and similar to genetic structure within the Gulf of Alaska and within the Bering Sea (Bentzen *et al.* 1998). Our analysis showed greater differentiation among spawning sites than among year classes. These results may have been biased by the distinction of the Cherry Point collections since there was a significant difference between year classes in Semiahmoo and overlap among Semiahmoo, Port Gamble and Northumberland, BC. We need additional year classes from sites other than Cherry Point to determine whether temporal and spatial patterns exist in Puget Sound that are similar to patterns in BC. As in the BC study (Beacham *et al.* 2002), the greatest differences were found in comparisons to San Francisco Bay herring.

Ware and Schweigert (2002) found that BC herring fit a metapopulation model where population dynamics depended upon climate regime. During warmer years, biomass and migration increased. Dispersal was density dependant so as herring population sizes

increased in Strait of Georgia and Prince Rupert, herring dispersed in a wave out to less populated regions. In Puget Sound, eighteen herring stocks have been identified based upon spawn timing, spawn location and phenotypic characters. While microsatellite analysis reveals inconsistent genetic differences among non-Cherry Point stocks, other regions of the herring genome may reveal stronger genetic differences paralleling the phenotypic and behavioral differences. Alternatively, herring may disperse throughout Puget Sound following a metapopulation model with location influencing behavior and phenotype. Inconsistencies in genetic relationships may reflect that migration rates are higher during warmer years. Inconsistencies may also indicate a "sweepstakes" style of recruitment where, although breeding numbers are large, chance events prevent most offspring from surviving to adulthood resulting in low numbers of effective spawners and high variance in genetic structure (Hedgecock 1994).

In summary, Cherry Point spawners are genetically distinct from other herring stocks identified in Puget Sound and Strait of Georgia. Differences in spawn timing are hypothesized to serve as a reproductive barrier promoting the genetic differentiation of the late-spawning Cherry Point herring. Although other herring stocks in Puget Sound are defined by discrete spawning locations and phenotypic differences, genetic relationships among them were less definitive. All herring stocks from Washington, British Columbia and Alaska were differentiated from San Francisco Bay herring and most stocks from Puget Sound were differentiated from Alaskan stocks.

## Acknowledgements

We thank Alice Pichahchy and Jennifer Von Bargen for their laboratory work and thank collectors from Alaska Department of Fish and Game and collectors Kurt Stick, Mark O'Toole and Pat MacAllister from WDFW. We collaborated on this project with Paul Bentzen at the University of Washington. Funding was provided through WDFW Marine Fish Division and State of Washington General Fund.

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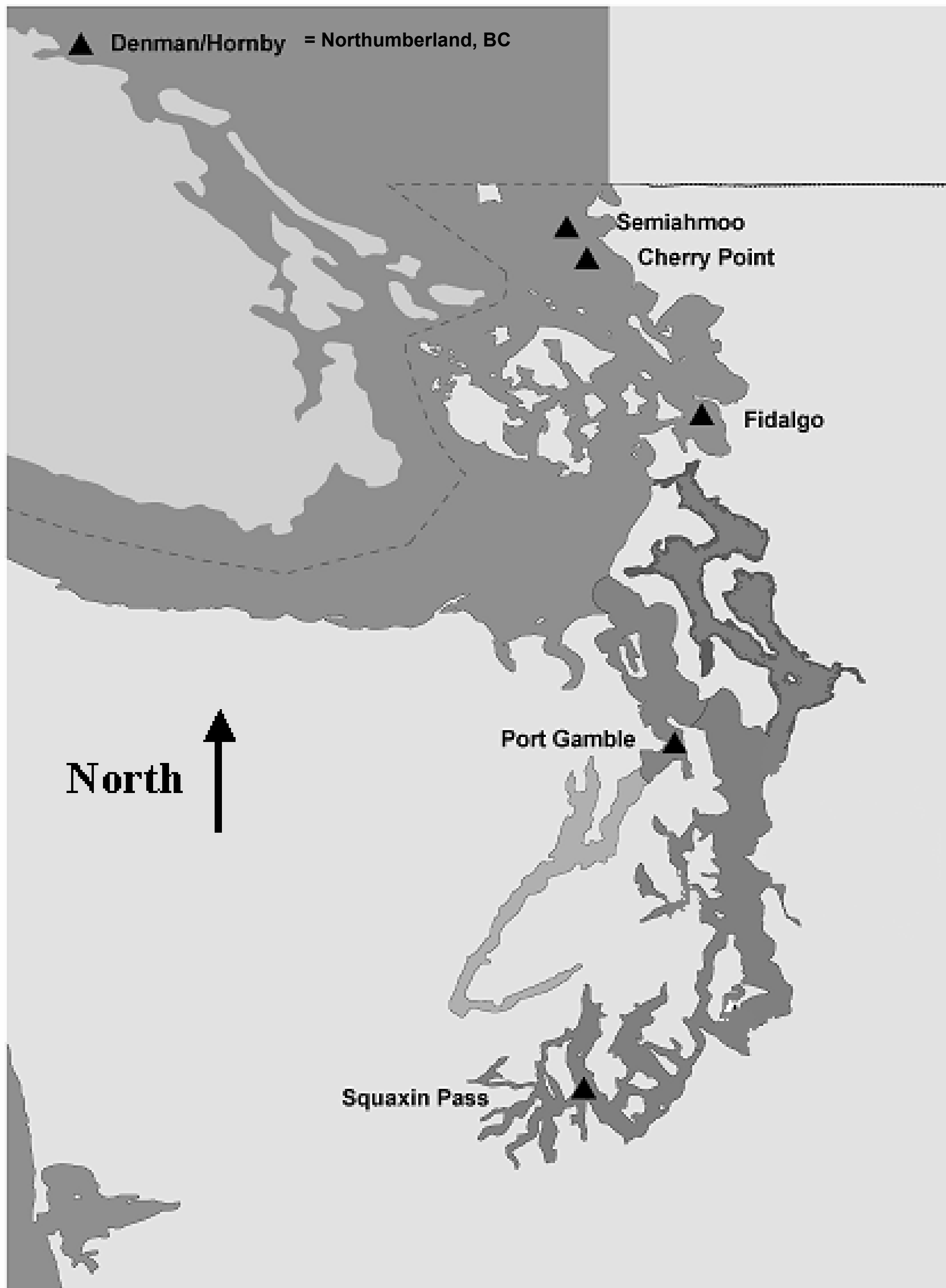
Figure 1. Map of collection sites for Pacific herring in Puget Sound and Strait of Georgia. Alaska sites are to the north of the map and San Francisco Bay is to the south of the map in California.



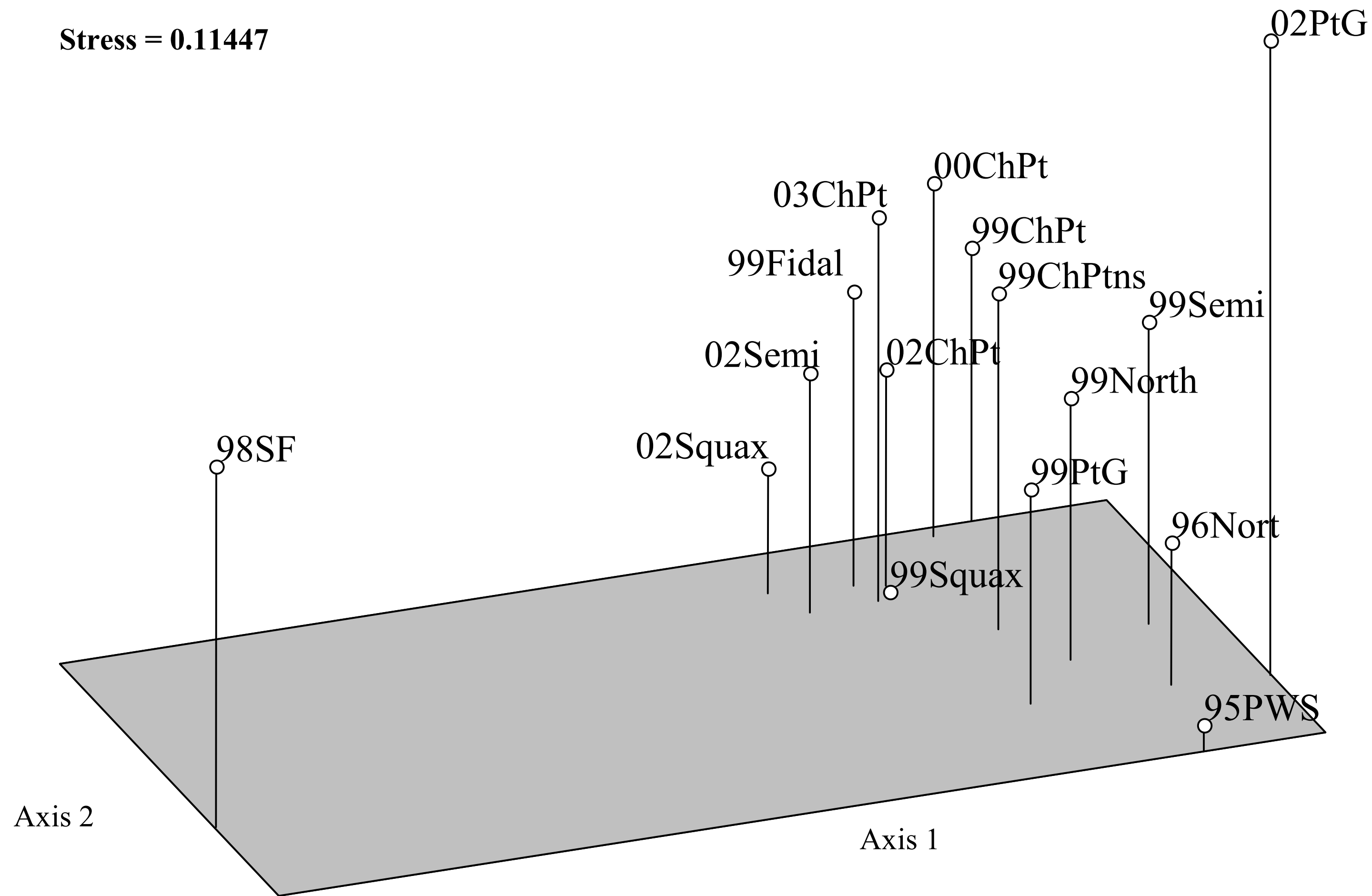



Figure 2. Multidimensional scaling plot of Nei's genetic chord distances (1983) among herring samples. Collections are abbreviated as follows: PtG = Port Gamble, ChPt = Cherry Point, ns = non-spawners, Nort = Norton Sound, PWS = Prince William Sound, Semi = Semiahmoo, North = Northumberland, Squax = Squaxin Pass, Fidal = Fidalgo, SF = San Francisco Bay.

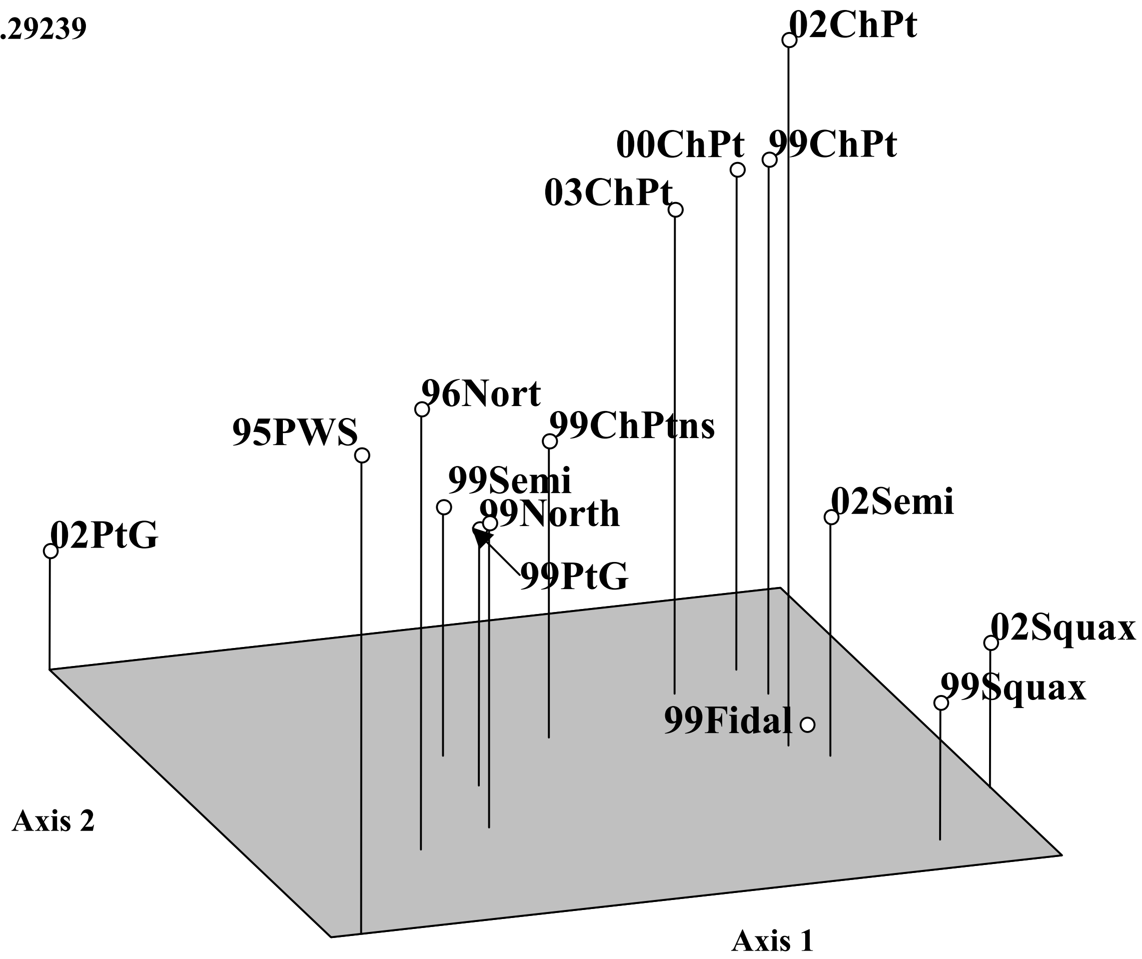



Figure 3. Multidimensional scaling plot of Nei's genetic chord distances (1983) among herring samples excluding San Francisco Bay collection. Abbreviations follow Figure 2.

Figure 4. Neighbor joining tree of Nei's genetic chord distances (1983) among herring collections. Numbers at the nodes indicate the percentage of 1000 trees in which collections beyond the nodes grouped together. Abbreviations follow Figure 2 except SqP = Squaxin Pass.

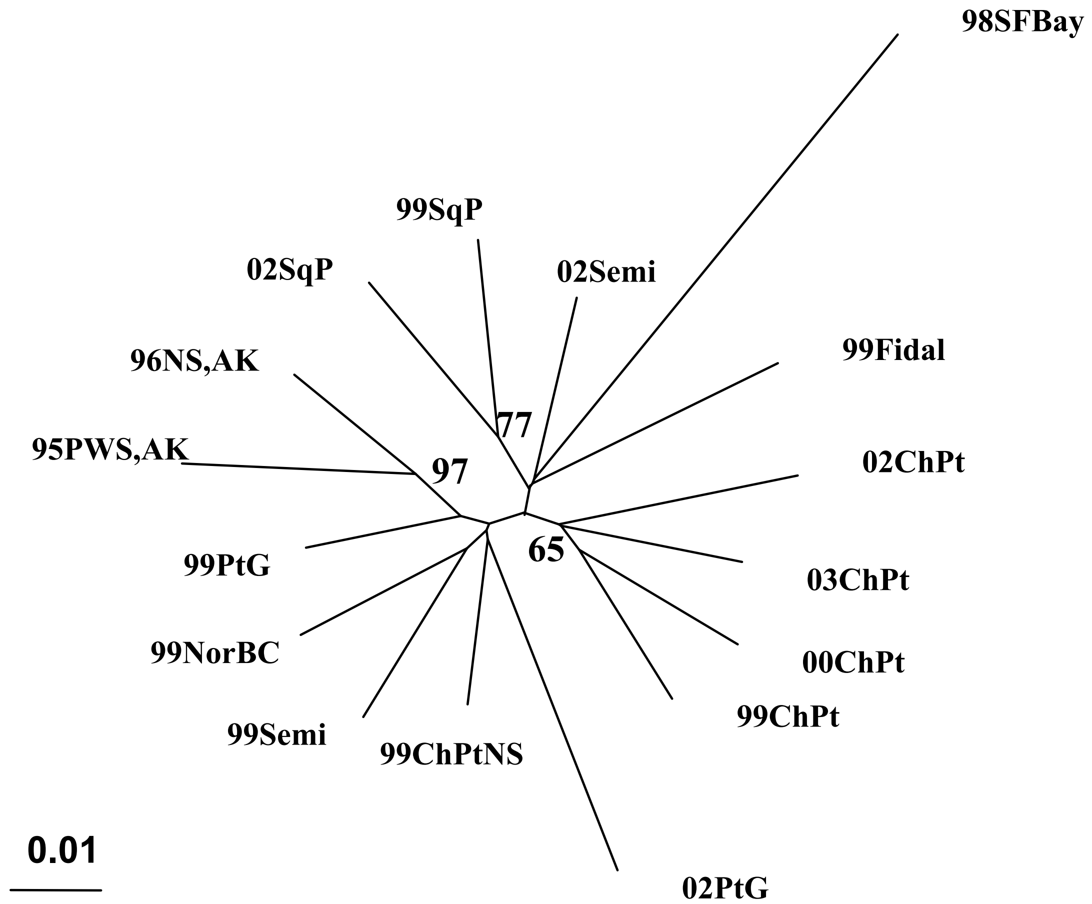


Figure 5. Principle components analysis plot of collections, excluding San Francisco Bay. Collections are plotted along axes one and three. Abbreviations follow Figure 2.

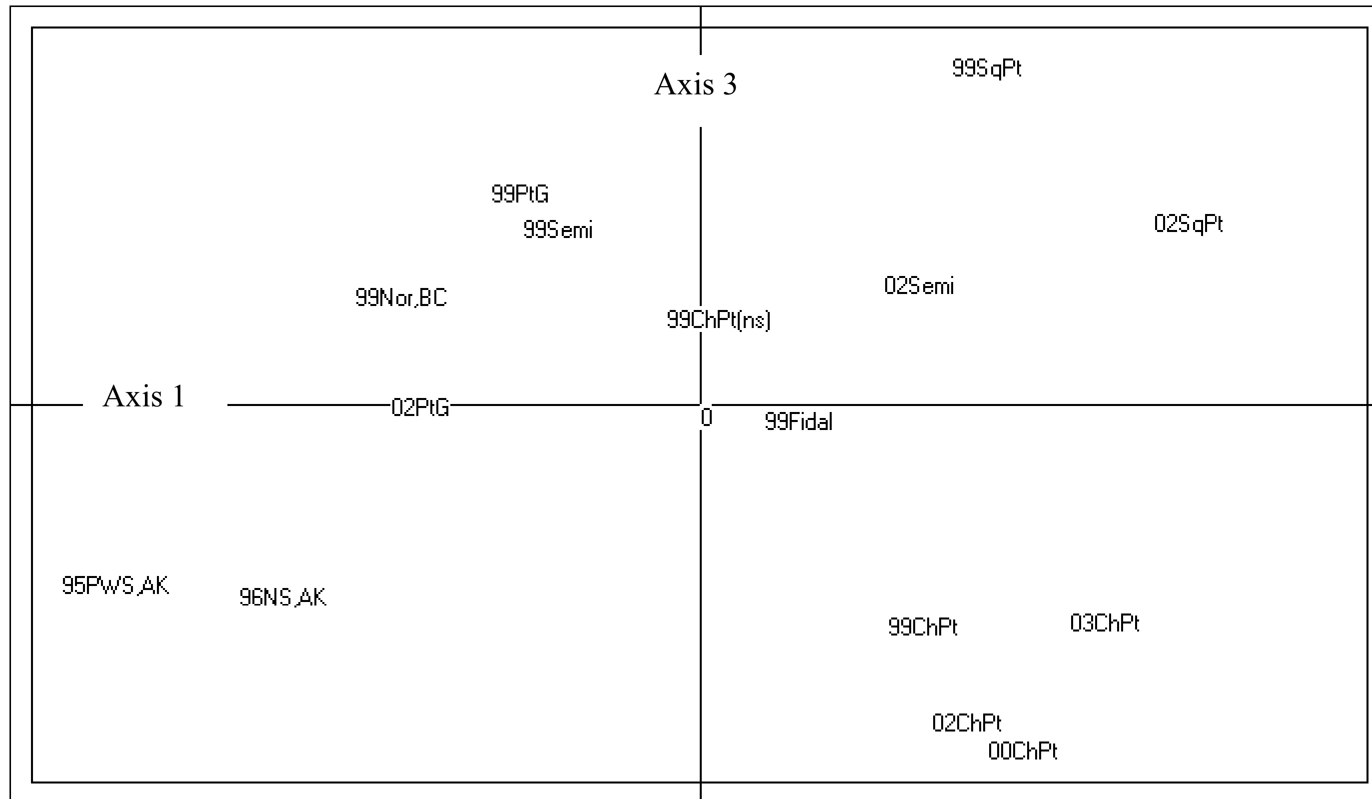


Table 1. Collections codes, year class and number of individuals collected and analyzed in collections and collection data. The "N > 5 loci" gives the number of individuals analyzed which amplified at five or more loci. The number of pairwise linkage disequilibria detected in the collection is in the "link" column. Observed heterozygosity over all loci is in the "Hobs" column, expected heterozygosity over all loci is in the "Hexp" column. Hardy Weinberg equilibrium *P*-values (HWE *P*, calculated without *Cha-8*) indicate whether collections deviated from Hardy Weinberg expectations with an excess of homozygotes, significant values are in bold type. Collections were tested for HWE using GENEPOP3.3 (Raymond and Rousset, 1995) with 100 batches and 2000 iterations. and allelic richness is in the "richness" column. Heterozygosity was calculated using MSA (Dieringer and Schlötterer 2002). Disequilibria was calculated with GENEPOP3.3 (Raymond and Rousset, 1995) using 200 batches and 2000 iterations. Allelic richness was calculated using FSTAT2.9.3 (Goudet 2001).

| Collection Code | Location | Date | N | N > 5 loci | link | Hobs | Hexp | HWE *P* | Allelic richness |
|---|---|---|---|---|---|---|---|---|---|
| 99OO | Cherry Point (non-spawners), WA | 28-Apr-99 | 96 | 95 | | 0.8199 | 0.8323 | **0** | 11.94 |
| 99ON | Cherry Point, WA | 28-Apr-99 | 96 | 95 | | 0.8211 | 0.8325 | 0.0456 | 12.37 |
| 00CA | Cherry Point, WA | 02-May-00 | 96 | 94 | 1 | 0.8054 | 0.8266 | 0.3102 | 12.27 |
| 02PA | Cherry Point, WA | 30-Apr-02 | 96 | 92 | 1 | 0.8150 | 0.8329 | 0.1385 | 12.87 |
| 03CG | Cherry Point, WA | 29-Apr-03 | 96 | 96 | | 0.8212 | 0.8252 | 0.4493 | 11.75 |
| 99OM | Semiahmoo, WA | 17-Feb-99 | 96 | 96 | | 0.8204 | 0.8320 | 0.2911 | 12.31 |
| 02PB | Semiahmoo, WA | 20-Feb-02 | 96 | 96 | | 0.8189 | 0.8328 | 0.3963 | 12.30 |
| 99OK | Squaxin Pass, WA | 01-Jan-99 | 96 | 96 | 1 | 0.8188 | 0.8301 | 0.0727 | 12.76 |
| 02PC | Squaxin Pass, WA | 16-Jan-02 | 96 | 96 | | 0.8193 | 0.8303 | 0.0339 | 12.21 |
| 99OL | Port Gamble, WA | 09-Feb-99 | 96 | 96 | 1 | 0.8190 | 0.8313 | 0.0013 | 12.50 |
| 02PD | Port Gamble, WA | 05-Feb-02 | 96 | 62 | 1 | 0.8230 | 0.8279 | 0.8004 | 10.67 |
| 99OQ | Fidalgo Bay, WA | 18-Feb-99 | 96 | 91 | 1 | 0.8202 | 0.8326 | **0.03** | 12.00 |
| 95ZF | Prince William Sound, AK | Fall, 96 | 96 | 93 | | 0.8223 | 0.8298 | **0** | 12.80 |
| 96ZG | Norton Sound, AK | Fall, 96 | 100 | 100 | | 0.8222 | 0.8315 | **0.0088** | 12.91 |
| 99OP | Northumberland, BC | 25-Feb-99 | 96 | 94 | 1 | 0.8204 | 0.8325 | **0.0276** | 12.29 |
| 98ZJ | San Francisco Bay, CA | NA | 67 | 61 | 6 | 0.8199 | 0.8301 | 0.1673 | 11.92 |
| | | | 1511 | 1453 | | | | | 12.24 |

Table 2. Loci information for each collection. Under each collection abbreviation are the $F_{IS}$ values at each locus (values significant before Bonferroni correction are underlined). The $F_{IS}$ row has $F_{IS}$ values for each collection calculated over all loci, the *P*-value for the overall $F_{IS}$ value (calculated without *Cha-8*) is in the *P*-value row. Values were calculated using FSTAT2.9.3 (Goudet 2001) with 192,000 randomizations. Collection names were abbreviated as follows: Cherry Point (ChPt), non-spawners (ns), Semiahmoo (Semi), Squaxin Point (SqP), Port Gamble (PtG), Fidalgo (Fidal), Prince William Sound (PWS), Norton Sound (NS), Northumberland (Nor), San Francisco (SF).

| | 99ChPt(ns) | 99ChPt | 00ChPt | 02ChPt | 03ChPt | 99Semi | 02Semi | 99SqP | 02SqP | 99PtG | 02PtG | 99Fidal | 95PWS | 96NS | 99Nor | 98SFBay |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cha-6* | -0.087 | -0.043 | -0.032 | -0.057 | -0.005 | -0.033 | -0.038 | 0.04 | -0.03 | -0.032 | -0.027 | 0.007 | 0.016 | 0.02 | 0.007 | 0.057 |
| *Cha-8* | 0.111 | 0.003 | 0.096 | 0.076 | 0.124 | 0.08 | 0.051 | 0.074 | 0.073 | 0.031 | -0.003 | 0.048 | 0.066 | 0.069 | 0.094 | 0.027 |
| *Cha-27* | 0.015 | -0.023 | -0.006 | 0.015 | -0.052 | 0.025 | 0.003 | 0.037 | -0.03 | 0.093 | -0.033 | 0.03 | 0.093 | 0.036 | -0.043 | -0.062 |
| *Cha-107* | 0.117 | 0.037 | 0.013 | -0.003 | -0.014 | -0.01 | -0.027 | 0.054 | -0.017 | -0.011 | 0.013 | -0.065 | 0.134 | 0.039 | 0.046 | 0.011 |
| *Cha-113* | 0.032 | -0.012 | 0.046 | -0.009 | -0.042 | -0.026 | -0.004 | 0.012 | 0.027 | 0.028 | -0.055 | -0.009 | 0.046 | -0.008 | -0.009 | 0.06 |
| *Cha-134* | 0.031 | 0.019 | 0.001 | -0.004 | 0.071 | 0.029 | 0.024 | 0.073 | 0.052 | 0.093 | -0.044 | 0.006 | 0.039 | 0.06 | 0.047 | 0.066 |
| *Cpa-A* | 0.058 | 0.068 | 0.093 | 0.042 | 0.024 | 0.039 | 0.09 | 0.092 | 0.03 | 0.004 | 0.041 | 0.079 | 0.035 | 0.04 | 0.036 | 0.034 |
| *Cpa-D* | 0.094 | 0.006 | 0.067 | -0.02 | -0.108 | 0.098 | -0.101 | -0.027 | 0.047 | 0.065 | -0.219 | -0.036 | -0.036 | -0.02 | 0.011 | 0.034 |
| *Cpa-H* | -0.004 | -0.01 | -0.045 | 0.059 | 0.009 | -0.042 | 0.012 | -0.024 | 0.039 | 0.034 | -0.039 | 0.029 | -0.013 | 0.023 | -0.013 | -0.009 |
| *Cpa-K* | -0.034 | 0.006 | -0.003 | 0.055 | 0.004 | -0.017 | -0.01 | -0.006 | -0.041 | 0.01 | 0.024 | 0.017 | -0.021 | 0.011 | 0.005 | -0.004 |
| *Cpa-172* | 0.029 | 0.018 | 0.038 | 0.113 | 0.057 | -0.063 | 0.021 | 0.027 | 0.021 | -0.011 | 0.061 | 0.014 | 0.049 | -0.005 | 0.031 | 0.106 |
| *Cpa-130* | 0.057 | 0.023 | 0.084 | -0.028 | -0.016 | 0.047 | 0.037 | -0.026 | -0.084 | 0.046 | -0.084 | 0.105 | 0.02 | 0.08 | -0.051 | -0.06 |
| $F_{IS}$ | 0.032 | 0.012 | 0.026 | 0.017 | -0.001 | 0.004 | 0.006 | 0.025 | 0.002 | 0.028 | -0.027 | 0.02 | 0.036 | 0.026 | 0.009 | 0.022 |
| *P*-value | **0.0036** | 0.1721 | 0.0169 | 0.0809 | 0.5252 | 0.3782 | 0.2959 | 0.015 | 0.4421 | **0.0067** | 0.9266 | 0.0591 | **0.001** | **0.0091** | 0.228 | 0.0704 |

Table 3. Information for multiplexes and loci including number of alleles in this study, size range (in basepairs), observed heterozygosity (Ho), repeat unit size (in basepairs), *P*-value for deviation from Hardy-Weinberg equilibrium (HWE), and primer sequences. Loci were tested for excesses of homozygotes using GENEPOP3.3 (Raymond and Rousset, 1995) with 100 batches and 2000 iterations. Values out of equilibrium are underlined and values out of equilibrium after Bonferroni corrections are in bold type. PCR's were conducted on a MJResearch PTC-200 thermocycler in 10 µl volumes employing 1 µl template with final concentrations of 1.5 mM $MgCl_2$, 0.05 units of *Taq* polymerase and 1X Promega PCR buffer.

| Multiplex | anneal T | cycles | dye | conc [uM] | locus | # alleles | range | Ho | repeat | HWE *P*-value | Primer | Primer sequence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cpa-A | 52 | 34 | hex | 0.3 | *Cha-6* | 22 | 158-258 | 0.724 | 4 | 0.8939 | *Cha-6* F | 5' - gtgtgagtttgctccaaa - 3' |
| | | | | | | | | | | | *Cha-6* R | 5' - gtttgtaccaatgaatgattacaa - 3' |
| | | | 6fam | 0.04 | *Cpa-172* | 23 | 175-263 | 0.86 | 4 | 0.0066 | *Cpa-172* F | 5' - gactcacaggttctcctcaaca - 3' |
| | | | | | | | | | | | *Cpa-172* R | 5' - tggagggattggaacattt - 3' |
| | | | ned | 0.2 | *Cha-27* | 19 | 97-209 | 0.876 | 4 | 0.0048 | *Cha-27* F | 5' - cacatttatcaatttctttg - 3' |
| | | | | | | | | | | | *Cha-27* R | 5' - gtttcagaaagagaatctaacctct - 3' |
| Cpa-B | 52 | 36 | 6fam | 0.07 | *Cha-107* | 30 | 108-168 | 0.872 | 2 | **0.0017** | *Cha-107* F | 5' - gcattacacagagaggaat - 3' |
| | | | | | | | | | | | *Cha-107* R | 5' - gtttagatacgcctctctcttt - 3' |
| | | | hex | 0.07 | *Cha-113* | 26 | 77-133 | 0.875 | 2 | 0.1359 | *Cha-113* F | 5' - cagtcagaaagaaggaga - 3' |
| | | | | | | | | | | | *Cha-113* R | 5' - gtttcctcctcgtgctcttt - 3' |
| | | | ned | 0.045 | *Cpa-130* | 27 | 204-308 | 0.82 | 4 | 0.1968 | *Cpa-130* F | 5' - atgatttttcgccttttgct - 3' |
| | | | | | | | | | | | *Cpa-130* R | 5' - cccagaaacaagagctaggc - 3' |
| Cpa-C | 52 | 32 | 6fam | 0.4 | *Cha-8* | 34 | 87-259 | 0.852 | 4 | **0** | *Cha-8* F | 5' - gatccttcttttaaggaaaa - 3' |
| | | | | | | | | | | | *Cha-8* R | 5' - gtttgacagaacttactatctcaga - 3' |
| | | | ned | 0.07 | *Cha-134* | 46 | 116-222 | 0.718 | 2 | **0.0001** | *Cha-134* F | 5' - cattctctacaaagggcatata - 3' |
| | | | | | | | | | | | *Cha-134* R | 5' - gtttcataccattgaatccagcta - 3' |
| Cpa-D | 58 | 34 | 6fam | 0.2 | *Cpa-D* | 21 | 136-232 | 0.597 | 4 | 0.1493 | *Cpa-D* F | 5' - ccatcctcatcaagaaagca - 3' |
| | | | | | | | | | | | *Cpa-D* R | 5' - ggtactttgacctctcctctcc - 3' |
| | | | ned | 0.25 | *Cpa-K* | 26 | 192-292 | 0.896 | 4 | 0.896 | *Cpa-K* F | 5' - gcgtttgtccataccacatt - 3' |
| | | | | | | | | | | | *Cpa-K* R | 5' - cagctctgaaaacccagaca - 3' |
| Cpa-E | 58 | 31 | hex | 0.1 | *Cpa-A* | 70 | 182-490 | 0.858 | 4 | **0** | *Cpa-A* F | 5' - tgattgggtccttttgaacat - 3' |
| | | | | | | | | | | | *Cpa-A* R | 5' - gcaatgactgacacagcaaa - 3' |
| | | | ned | 0.1 | *Cpa-H* | 23 | 228-316 | 0.929 | 4 | 0.2158 | *Cpa-H* F | 5' - tgtccagtaaaacatgcctga - 3' |
| | | | | | | | | | | | *Cpa-H* R | 5' - gctccgttctctttcttgct - 3' |

Table 4. *P*-values for pairwise tests of genotypic differentiation over all loci (below diagonal) and number of significantly different pairwise genotypic tests between collections (above diagonal). Significant *P*-values are in bold type. In upper matrix the first value is the number of pairwise tests with $P < 0.05$ and value in parentheses is the number of pairwise tests with $P < 0.0004$, the corrected value for multiple simultaneous tests (0.05/120). Cells in lower matrix containing "h.s." indicate undefined but significant *P*-values: Chi square value for tests over all loci was infinity and *P*-value was highly significant. Chi square tests were conducted using GENEPOP3.3 (Raymond and Rousset, 1995) with 300 batches and 2000 iterations. Abbreviations follow Table 2, with Alaska = AK and British Columbia = BC.

| | 99ChPt(ns) | 99ChPt | 00ChPt | 02ChPt | 03ChPt | 99Semi | 02Semi | 99SqP | 02SqP | 99PtG | 02PtG | 99Fidal | 95PWS,AK | 96NS,AK | 99Nor,BC | 98SFBay |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | - | 2 (0) | 4 (0) | 3 (0) | 4 (1) | 0 (0) | 4 (1) | 4 (1) | 2 (1) | 1 (1) | 2 (0) | 3 (1) | 5 (0) | 3 (0) | 2 (0) | 7 (1) |
| 99ChPt | 0.04939 | - | 0 (0) | 0 (0) | 1 (0) | 3 (0) | 3 (1) | 2 (1) | 5 (3) | 3 (0) | 3 (0) | 4 (1) | 6 (1) | 4 (2) | 4 (2) | 6 (4) |
| 00ChPt | 0.00073 | 0.74536 | - | 0 (0) | 0 (0) | 5 (1) | 5 (1) | 4 (1) | 4 (1) | 3 (2) | 3 (1) | 3 (1) | 5 (2) | 4 (2) | 4 (3) | 5 (2) |
| 02ChPt | 0.00517 | 0.56806 | 0.31465 | - | 0 (0) | 7 (1) | 3 (1) | 4 (1) | 3 (1) | 3 (2) | 3 (1) | 2 (1) | 3 (2) | 2 (2) | 4 (2) | 6 (4) |
| 03ChPt | 0.0008 | 0.37863 | 0.58249 | 0.51132 | - | 7 (1) | 2 (0) | 4 (2) | 2 (1) | 5 (1) | 3 (1) | 4 (1) | 5 (3) | 4 (2) | 3 (2) | 6 (3) |
| 99Semi | 0.44811 | **0.00038** | **h.s.** | **h.s.** | **h.s.** | - | 3 (1) | 4 (2) | 5 (2) | 1 (0) | 1 (0) | 4 (1) | 4 (1) | 5 (0) | 2 (0) | 8 (3) |
| 02Semi | **h.s.** | **h.s.** | **0.00001** | **h.s.** | **0.00015** | **h.s.** | - | 5 (1) | 3 (1) | 2 (1) | 3 (1) | 3 (0) | 5 (2) | 3 (2) | 3 (2) | 6 (2) |
| 99SqP | **0.00001** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **0.00001** | - | 0 (0) | 2 (1) | 3 (2) | 5 (1) | 4 (2) | 4 (2) | 4 (1) | 7 (2) |
| 02SqP | **h.s.** | **h.s.** | **0.00001** | **0.00006** | **h.s.** | **h.s.** | **h.s.** | 0.23206 | - | 2 (1) | 4 (1) | 5 (0) | 4 (3) | 4 (1) | 2 (1) | 5 (2) |
| 99PtG | 0.11549 | **0.00001** | **h.s.** | **0.00006** | **h.s.** | 0.20726 | **h.s.** | **0.00005** | **h.s.** | - | 3 (0) | 4 (1) | 3 (0) | 2 (0) | 1 (0) | 4 (2) |
| 02PtG | 0.10197 | 0.00097 | **h.s.** | **0.00051** | **h.s.** | 0.13371 | **h.s.** | **h.s.** | **h.s.** | 0.02798 | - | 3 (1) | 4 (1) | 2 (0) | 2 (0) | 4 (3) |
| 99Fidal | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | 0.00662 | **0.00001** | **h.s.** | **h.s.** | **h.s.** | - | 5 (1) | 3 (2) | 3 (1) | 7 (3) |
| 95PWS,AK | **0.00001** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **0.00008** | **h.s.** | **h.s.** | **h.s.** | 0.00993 | 0.00138 | **h.s.** | - | 0 (0) | 5 (0) | 7 (3) |
| 96NS,AK | 0.00049 | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **0.00005** | **h.s.** | **h.s.** | **h.s.** | 0.05159 | 0.10598 | **h.s.** | 0.83574 | - | 1 (0) | 6 (3) |
| 99Nor,BC | 0.02848 | **h.s.** | **h.s.** | **h.s.** | **h.s.** | 0.06714 | **h.s.** | **h.s.** | **h.s.** | 0.09997 | 0.08842 | **h.s.** | 0.0004 | 0.05765 | - | 5 (3) |
| 98SFBay | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | **h.s.** | - |

Table 5. AMOVA results (averaged over 11 loci) from Arlequin 2.001 (Schneider *et al.* 2000). Partitioning of molecular variance was explored with collections in a single group and divided by site and by year class with 15,000 iterations.

| Groupings | df | Source of variation | Variance | Fixation index | *P* value |
|---|---|---|---|---|---|
| All collections included | 2890 | within collections | 0.0236 | $F_{ST} = 0.00513$ | $P < 0.005$ |
| Divided by site | 7 | among sites | 0.02098 | $F_{CT} = 0.00455$ | $P < 0.005$ |
| | 8 | among years within sites | 0.00523 | $F_{SC} = 0.00114$ | $P < 0.005$ |
| | 2890 | within collections | 4.5846 | $F_{ST} = 0.00568$ | $P < 0.005$ |
| Divided by year | 6 | among years | 0.0098 | $F_{CT} = 0.00212$ | $P < 0.005$ |
| | 9 | among sites within year | 0.01607 | $F_{SC} = 0.00349$ | $P < 0.005$ |
| | 2890 | within collections | 4.5846 | $F_{ST} = 0.00561$ | $P < 0.005$ |
| Within Puget Sound and Strait of Georgia | 2397 | within collections | 0.0164 | $F_{ST} = 0.00358$ | $P < 0.005$ |
| Divided by site | 5 | among sites | 0.01261 | $F_{CT} = 0.00275$ | $P < 0.005$ |
| | 7 | among years within sites | 0.00604 | $F_{SC} = 0.00132$ | $P < 0.005$ |
| | 2385 | within collections | 4.573 | $F_{ST} = 0.00406$ | $P < 0.005$ |
| Divided by year | 3 | among years | 0.0057 | $F_{CT} = 0.00012$ | $P < 0.005$ |
| | 9 | among sites within year | 0.016 | $F_{SC} = 0.00349$ | $P < 0.005$ |
| | 2385 | within collections | 4.573 | $F_{ST} = 0.00361$ | $P < 0.005$ |

Appendix I. Allele table. Locus name is in the first column and alleles are identified by size in basepairs.

| *Cha-27* | 97 | 105 | 109 | 117 | 121 | 125 | 129 | 133 | 141 | 145 | 153 | 157 | 165 | 169 | 173 | 177 | 181 | 193 | 209 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 3 | 0 | 90 | 14 | 10 | 0 | 22 | 11 | 6 | 3 | 15 | 6 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 184 |
| 99ChPt | 1 | 0 | 81 | 35 | 6 | 0 | 13 | 10 | 4 | 0 | 6 | 7 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 166 |
| 00ChPt | 3 | 0 | 96 | 23 | 4 | 0 | 16 | 12 | 6 | 1 | 8 | 5 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 180 |
| 02CHPt | 1 | 0 | 88 | 34 | 6 | 1 | 15 | 11 | 5 | 0 | 6 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 170 |
| 03ChPt | 1 | 0 | 93 | 25 | 11 | 0 | 15 | 14 | 1 | 2 | 7 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 174 |
| 99Semi | 1 | 0 | 89 | 20 | 20 | 0 | 13 | 6 | 16 | 2 | 11 | 2 | 7 | 0 | 0 | 2 | 1 | 0 | 0 | 190 |
| 02Semi | 0 | 1 | 90 | 12 | 28 | 0 | 8 | 16 | 12 | 4 | 9 | 4 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 192 |
| 99SqPass | 2 | 0 | 112 | 6 | 21 | 0 | 9 | 6 | 10 | 2 | 15 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 186 |
| 02SqPass | 1 | 0 | 92 | 8 | 15 | 0 | 8 | 11 | 8 | 3 | 10 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 158 |
| 99PtGam | 3 | 0 | 88 | 13 | 14 | 0 | 16 | 9 | 15 | 3 | 15 | 3 | 6 | 0 | 0 | 2 | 0 | 1 | 0 | 188 |
| 02PtGam | 0 | 0 | 19 | 4 | 5 | 0 | 10 | 0 | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 44 |
| 99Fidalgo | 2 | 0 | 59 | 5 | 29 | 0 | 25 | 16 | 7 | 3 | 8 | 1 | 6 | 1 | 0 | 2 | 0 | 0 | 0 | 164 |
| 95PrinceW | 2 | 0 | 35 | 9 | 7 | 0 | 16 | 4 | 20 | 3 | 16 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 116 |
| 96NortonS | 1 | 0 | 82 | 14 | 4 | 0 | 27 | 7 | 24 | 5 | 16 | 3 | 3 | 0 | 0 | 1 | 0 | 0 | 1 | 188 |
| 99Northum | 5 | 1 | 85 | 7 | 12 | 0 | 25 | 7 | 16 | 7 | 11 | 0 | 5 | 0 | 0 | 2 | 1 | 0 | 0 | 184 |
| 98SFBay | 5 | 0 | 59 | 5 | 7 | 0 | 8 | 7 | 9 | 3 | 7 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 114 |
| Total | 31 | 2 | 1258 | 234 | 199 | 1 | 246 | 147 | 163 | 42 | 161 | 37 | 54 | 5 | 2 | 11 | 3 | 1 | 1 | 2598 |

| *Cha-113* | 77 | 83 | 85 | 87 | 89 | 91 | 93 | 95 | 97 | 99 | 101 | 103 | 105 | 107 | 109 | 111 | 113 | 115 | 117 | 119 | 121 | 123 | 125 | 129 | 131 | 133 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 7 | 7 | 1 | 11 | 23 | 27 | 14 | 35 | 36 | 7 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 178 |
| 99ChPt | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 11 | 3 | 7 | 22 | 31 | 14 | 32 | 30 | 9 | 5 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 170 |
| 00ChPt | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 7 | 2 | 9 | 29 | 28 | 9 | 37 | 29 | 8 | 5 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 174 |
| 02CHPt | 0 | 0 | 1 | 1 | 1 | 0 | 5 | 7 | 6 | 8 | 4 | 20 | 34 | 18 | 29 | 34 | 2 | 5 | 3 | 0 | 2 | 0 | 2 | 2 | 0 | 0 | 184 |
| 03ChPt | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 7 | 6 | 8 | 23 | 27 | 17 | 41 | 37 | 4 | 5 | 4 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 188 |
| 99Semi | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4 | 11 | 3 | 11 | 21 | 22 | 14 | 30 | 32 | 7 | 5 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 164 |
| 02Semi | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 2 | 12 | 3 | 12 | 20 | 23 | 17 | 24 | 39 | 9 | 3 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 174 |
| 99SqPass | 0 | 0 | 0 | 0 | 3 | 0 | 4 | 4 | 10 | 7 | 10 | 16 | 36 | 15 | 33 | 19 | 3 | 4 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 168 |
| 02SqPass | 0 | 0 | 0 | 0 | 3 | 0 | 7 | 5 | 12 | 5 | 10 | 22 | 29 | 13 | 31 | 35 | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 178 |
| 99PtGam | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 7 | 9 | 7 | 9 | 19 | 31 | 20 | 21 | 39 | 4 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 176 |
| 02PtGam | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 7 | 4 | 11 | 19 | 24 | 7 | 30 | 22 | 2 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 134 |
| 99Fidalgo | 1 | 0 | 0 | 0 | 3 | 0 | 4 | 1 | 16 | 5 | 7 | 20 | 23 | 20 | 29 | 29 | 7 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 170 |
| 95PrinceW | 0 | 0 | 0 | 0 | 1 | 2 | 4 | 4 | 12 | 7 | 11 | 22 | 31 | 19 | 29 | 29 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 178 |

| *Cha-113* cont | 77 | 83 | 85 | 87 | 89 | 91 | 93 | 95 | 97 | 99 | 101 | 103 | 105 | 107 | 109 | 111 | 113 | 115 | 117 | 119 | 121 | 123 | 125 | 129 | 131 | 133 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 96NortonS | 0 | 0 | 0 | 0 | 1 | 1 | 5 | 3 | 11 | 8 | 9 | 17 | 30 | 8 | 47 | 32 | 6 | 5 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 186 |
| 99Northum | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 3 | 8 | 4 | 8 | 26 | 35 | 12 | 32 | 29 | 5 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 170 |
| 98SFBay | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 5 | 4 | 5 | 4 | 14 | 16 | 13 | 14 | 15 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| Total | 1 | 1 | 2 | 1 | 14 | 7 | 58 | 59 | 150 | 78 | 141 | 333 | 447 | 230 | 494 | 486 | 83 | 57 | 26 | 3 | 7 | 1 | 7 | 4 | 1 | 1 | 2692 |

| *Cha-107* | 108 | 112 | 114 | 116 | 118 | 120 | 122 | 124 | 126 | 128 | 130 | 132 | 134 | 136 | 138 | 140 | 142 | 144 | 146 | 148 | 150 | 152 | 154 | 156 | 158 | 160 | 162 | 164 | 166 | 168 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 0 | 0 | 0 | 8 | 0 | 2 | 1 | 21 | 26 | 32 | 16 | 27 | 16 | 3 | 5 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 11 | 1 | 3 | 3 | 0 | 178 |
| 99ChPt | 0 | 0 | 0 | 0 | 9 | 2 | 1 | 5 | 20 | 14 | 39 | 5 | 27 | 9 | 3 | 6 | 1 | 2 | 1 | 0 | 2 | 2 | 1 | 0 | 0 | 11 | 3 | 4 | 9 | 0 | 176 |
| 00ChPt | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 6 | 20 | 14 | 28 | 10 | 15 | 4 | 2 | 2 | 3 | 0 | 0 | 1 | 0 | 5 | 1 | 0 | 0 | 18 | 1 | 7 | 9 | 1 | 158 |
| 02CHPt | 1 | 1 | 0 | 1 | 9 | 0 | 3 | 1 | 22 | 19 | 32 | 7 | 16 | 7 | 3 | 3 | 2 | 2 | 0 | 2 | 4 | 0 | 1 | 0 | 0 | 25 | 3 | 8 | 10 | 0 | 182 |
| 03ChPt | 0 | 0 | 0 | 0 | 15 | 0 | 1 | 5 | 13 | 20 | 41 | 10 | 17 | 9 | 1 | 2 | 4 | 3 | 1 | 0 | 5 | 0 | 0 | 0 | 0 | 18 | 2 | 4 | 8 | 1 | 180 |
| 99Semi | 0 | 1 | 1 | 0 | 10 | 1 | 6 | 2 | 17 | 20 | 27 | 9 | 29 | 11 | 5 | 8 | 5 | 0 | 1 | 2 | 4 | 1 | 0 | 0 | 0 | 4 | 1 | 1 | 2 | 0 | 168 |
| 02Semi | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 5 | 11 | 20 | 20 | 11 | 16 | 13 | 1 | 3 | 4 | 0 | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 34 | 6 | 13 | 19 | 0 | 186 |
| 99SqPass | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 4 | 13 | 11 | 42 | 10 | 22 | 7 | 1 | 4 | 0 | 3 | 0 | 1 | 0 | 8 | 5 | 2 | 0 | 16 | 5 | 5 | 5 | 0 | 168 |
| 02SqPass | 0 | 0 | 0 | 0 | 5 | 0 | 2 | 2 | 6 | 8 | 52 | 4 | 18 | 9 | 1 | 1 | 0 | 0 | 2 | 2 | 0 | 7 | 7 | 0 | 1 | 32 | 6 | 12 | 3 | 0 | 180 |
| 99PtGam | 0 | 0 | 0 | 0 | 2 | 0 | 4 | 2 | 18 | 26 | 24 | 13 | 39 | 21 | 7 | 8 | 3 | 2 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 9 | 0 | 3 | 4 | 0 | 192 |
| 02PtGam | 0 | 0 | 0 | 0 | 6 | 2 | 1 | 0 | 25 | 25 | 17 | 4 | 23 | 10 | 9 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 2 | 1 | 4 | 0 | 136 |
| 99Fidalgo | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 3 | 14 | 8 | 31 | 5 | 19 | 7 | 1 | 4 | 0 | 1 | 0 | 2 | 1 | 4 | 0 | 0 | 0 | 32 | 3 | 7 | 14 | 0 | 164 |
| 95PrinceW | 0 | 0 | 0 | 1 | 5 | 0 | 6 | 5 | 33 | 26 | 20 | 12 | 28 | 9 | 6 | 3 | 1 | 2 | 2 | 0 | 1 | 4 | 4 | 1 | 0 | 11 | 0 | 0 | 0 | 0 | 180 |
| 96NortonS | 0 | 0 | 0 | 1 | 2 | 1 | 2 | 5 | 35 | 36 | 20 | 16 | 14 | 15 | 4 | 4 | 3 | 1 | 0 | 2 | 0 | 2 | 3 | 4 | 0 | 3 | 2 | 2 | 3 | 0 | 180 |
| 99Northum | 0 | 0 | 0 | 0 | 8 | 2 | 5 | 5 | 24 | 35 | 23 | 9 | 26 | 12 | 0 | 5 | 7 | 2 | 0 | 0 | 3 | 1 | 1 | 0 | 0 | 3 | 2 | 2 | 1 | 0 | 176 |
| 98SFBay | 0 | 0 | 0 | 0 | 4 | 0 | 1 | 0 | 5 | 9 | 19 | 6 | 5 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 24 | 12 | 16 | 3 | 2 | 112 |
| Total | 1 | 3 | 1 | 3 | 101 | 14 | 36 | 51 | 297 | 317 | 467 | 147 | 341 | 161 | 47 | 59 | 36 | 18 | 7 | 14 | 25 | 41 | 26 | 8 | 2 | 255 | 49 | 88 | 97 | 4 | 2716 |

| *Cha-134* | 116 | 118 | 120 | 124 | 126 | 128 | 130 | 132 | 134 | 136 | 138 | 140 | 142 | 144 | 146 | 148 | 150 | 152 | 154 | 156 | 158 | 160 | 162 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 1 | 0 | 0 | 0 | 2 | 0 | 28 | 12 | 6 | 20 | 12 | 28 | 18 | 21 | 9 | 11 | 4 | 0 | 4 | 1 | 2 | 2 | 4 |
| 99ChPt | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 8 | 7 | 16 | 11 | 23 | 12 | 15 | 14 | 11 | 5 | 1 | 1 | 4 | 3 | 2 | 0 |
| 00ChPt | 1 | 0 | 0 | 3 | 0 | 2 | 24 | 7 | 15 | 25 | 14 | 29 | 7 | 8 | 9 | 10 | 2 | 1 | 2 | 2 | 2 | 1 | 2 |
| 02CHPt | 0 | 0 | 0 | 0 | 2 | 2 | 31 | 7 | 6 | 12 | 14 | 24 | 7 | 15 | 6 | 8 | 3 | 2 | 1 | 4 | 2 | 1 | 2 |
| 03ChPt | 0 | 2 | 0 | 0 | 1 | 2 | 28 | 4 | 18 | 20 | 12 | 27 | 20 | 15 | 6 | 5 | 2 | 1 | 1 | 3 | 1 | 3 | 1 |
| 99Semi | 0 | 0 | 0 | 0 | 3 | 1 | 25 | 4 | 10 | 15 | 17 | 30 | 16 | 16 | 7 | 12 | 2 | 3 | 0 | 2 | 2 | 3 | 2 |
| 02Semi | 0 | 2 | 0 | 1 | 0 | 1 | 30 | 11 | 11 | 21 | 12 | 26 | 13 | 18 | 8 | 13 | 4 | 4 | 2 | 2 | 3 | 1 | 3 |
| 99SqPass | 1 | 1 | 0 | 3 | 0 | 0 | 34 | 14 | 14 | 16 | 13 | 20 | 14 | 15 | 13 | 6 | 4 | 2 | 2 | 1 | 3 | 0 | 0 |
| 02SqPass | 0 | 1 | 0 | 1 | 0 | 1 | 30 | 8 | 11 | 17 | 18 | 17 | 22 | 14 | 13 | 7 | 2 | 1 | 4 | 0 | 2 | 0 | 0 |

| *Cha-134* cont | 116 | 118 | 120 | 124 | 126 | 128 | 130 | 132 | 134 | 136 | 138 | 140 | 142 | 144 | 146 | 148 | 150 | 152 | 154 | 156 | 158 | 160 | 162 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99PtGam | 0 | 1 | 0 | 0 | 0 | 2 | 31 | 10 | 15 | 22 | 14 | 21 | 15 | 20 | 6 | 8 | 5 | 2 | 2 | 0 | 1 | 0 | 2 |
| 02PtGam | 0 | 0 | 0 | 0 | 0 | 1 | 21 | 4 | 9 | 14 | 9 | 18 | 8 | 8 | 2 | 6 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| 99Fidalgo | 0 | 1 | 0 | 1 | 0 | 0 | 18 | 5 | 4 | 30 | 11 | 20 | 6 | 15 | 6 | 10 | 1 | 2 | 2 | 1 | 0 | 0 | 2 |
| 95PrinceW | 0 | 0 | 1 | 0 | 2 | 1 | 29 | 8 | 11 | 22 | 14 | 22 | 16 | 19 | 10 | 4 | 4 | 1 | 1 | 0 | 1 | 0 | 1 |
| 96NortonS | 0 | 0 | 1 | 0 | 1 | 1 | 27 | 5 | 15 | 24 | 19 | 21 | 14 | 28 | 5 | 12 | 5 | 1 | 1 | 1 | 2 | 0 | 2 |
| 99Northum | 0 | 1 | 0 | 2 | 2 | 1 | 24 | 7 | 18 | 21 | 11 | 25 | 12 | 11 | 10 | 13 | 2 | 2 | 4 | 4 | 1 | 0 | 2 |
| 98SFBay | 0 | 1 | 0 | 0 | 0 | 0 | 16 | 2 | 13 | 6 | 14 | 21 | 8 | 11 | 7 | 5 | 3 | 1 | 5 | 0 | 0 | 3 | 0 |
| Total | 3 | 10 | 2 | 11 | 13 | 15 | 426 | 116 | 183 | 301 | 215 | 372 | 208 | 249 | 131 | 141 | 49 | 24 | 33 | 25 | 25 | 16 | 23 |

| *Cha-134* cont | 164 | 166 | 168 | 170 | 172 | 174 | 176 | 178 | 180 | 182 | 184 | 188 | 190 | 192 | 194 | 198 | 200 | 202 | 204 | 206 | 210 | 214 | 222 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 188 |
| 99ChPt | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 166 |
| 00ChPt | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 170 |
| 02CHPt | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 154 |
| 03ChPt | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 178 |
| 99Semi | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 180 |
| 02Semi | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 192 |
| 99SqPass | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 182 |
| 02SqPass | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 174 |
| 99PtGam | 2 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 188 |
| 02PtGam | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 102 |
| 99Fidalgo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 136 |
| 95PrinceW | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 178 |
| 96NortonS | 2 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 192 |
| 99Northum | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 180 |
| 98SFBay | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 118 |
| Total | 12 | 11 | 8 | 8 | 1 | 6 | 4 | 5 | 2 | 2 | 4 | 2 | 2 | 2 | 1 | 3 | 2 | 3 | 4 | 1 | 1 | 1 | 2 | 2678 |

| *Cpa-D* | 136 | 140 | 152 | 156 | 164 | 168 | 172 | 176 | 180 | 184 | 188 | 192 | 196 | 200 | 204 | 208 | 212 | 220 | 224 | 228 | 232 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 1 | 0 | 0 | 0 | 103 | 0 | 20 | 10 | 31 | 5 | 12 | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 188 |
| 99ChPt | 0 | 2 | 0 | 0 | 1 | 96 | 0 | 21 | 14 | 23 | 2 | 3 | 3 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 168 |
| 00ChPt | 0 | 1 | 0 | 0 | 0 | 111 | 0 | 10 | 12 | 30 | 7 | 7 | 2 | 2 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 186 |
| 02CHPt | 1 | 0 | 1 | 0 | 0 | 104 | 1 | 12 | 9 | 17 | 5 | 3 | 6 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 162 |
| 03ChPt | 0 | 1 | 0 | 0 | 0 | 116 | 2 | 5 | 10 | 20 | 3 | 5 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 168 |

| *Cpa-D* cont | 136 | 140 | 152 | 156 | 164 | 168 | 172 | 176 | 180 | 184 | 188 | 192 | 196 | 200 | 204 | 208 | 212 | 220 | 224 | 228 | 232 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99Semi | 0 | 1 | 0 | 0 | 0 | 99 | 0 | 24 | 10 | 23 | 10 | 13 | 1 | 1 | 0 | 1 | 5 | 0 | 0 | 0 | 0 | 188 |
| 02Semi | 0 | 4 | 0 | 0 | 0 | 130 | 0 | 13 | 8 | 19 | 5 | 3 | 1 | 0 | 1 | 1 | 2 | 2 | 0 | 0 | 1 | 190 |
| 99SqPass | 0 | 0 | 0 | 0 | 0 | 107 | 0 | 10 | 15 | 23 | 5 | 9 | 6 | 1 | 3 | 3 | 2 | 0 | 0 | 0 | 0 | 184 |
| 02SqPass | 0 | 0 | 0 | 0 | 0 | 123 | 2 | 16 | 12 | 19 | 7 | 3 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 186 |
| 99PtGam | 0 | 0 | 0 | 0 | 0 | 112 | 3 | 18 | 17 | 15 | 11 | 6 | 3 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 192 |
| 02PtGam | 0 | 0 | 0 | 0 | 0 | 79 | 0 | 10 | 9 | 15 | 3 | 5 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 124 |
| 99Fidalgo | 0 | 1 | 0 | 0 | 0 | 111 | 2 | 9 | 9 | 15 | 6 | 10 | 6 | 1 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 174 |
| 95PrinceW | 0 | 0 | 0 | 0 | 0 | 109 | 2 | 8 | 14 | 21 | 11 | 9 | 3 | 2 | 1 | 3 | 1 | 0 | 1 | 1 | 0 | 186 |
| 96NortonS | 1 | 1 | 0 | 0 | 0 | 124 | 3 | 9 | 8 | 21 | 10 | 8 | 5 | 2 | 1 | 6 | 0 | 0 | 1 | 0 | 0 | 200 |
| 99Northum | 0 | 2 | 0 | 1 | 0 | 108 | 3 | 10 | 12 | 27 | 5 | 7 | 5 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 184 |
| 98SFBay | 0 | 0 | 0 | 0 | 0 | 80 | 1 | 8 | 4 | 12 | 1 | 2 | 5 | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 0 | 118 |
| Total | 2 | 14 | 1 | 1 | 1 | 1712 | 19 | 203 | 173 | 331 | 96 | 105 | 55 | 19 | 12 | 28 | 19 | 3 | 2 | 1 | 1 | 2798 |

| *Cpa-K* | 192 | 196 | 200 | 204 | 208 | 212 | 216 | 220 | 224 | 228 | 232 | 236 | 240 | 244 | 248 | 252 | 256 | 260 | 264 | 268 | 272 | 276 | 280 | 284 | 288 | 292 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 0 | 2 | 10 | 17 | 20 | 28 | 23 | 22 | 26 | 9 | 10 | 2 | 3 | 8 | 1 | 3 | 1 | 0 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 190 |
| 99ChPt | 0 | 0 | 0 | 13 | 14 | 19 | 24 | 18 | 27 | 26 | 14 | 8 | 5 | 3 | 5 | 2 | 1 | 1 | 0 | 2 | 3 | 1 | 0 | 0 | 2 | 0 | 188 |
| 00ChPt | 0 | 0 | 3 | 11 | 15 | 19 | 22 | 20 | 25 | 26 | 8 | 10 | 4 | 4 | 6 | 3 | 3 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 182 |
| 02CHPt | 0 | 0 | 2 | 3 | 13 | 9 | 15 | 13 | 20 | 19 | 6 | 4 | 1 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 108 |
| 03ChPt | 0 | 0 | 1 | 12 | 13 | 12 | 14 | 29 | 20 | 20 | 9 | 3 | 3 | 3 | 5 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 148 |
| 99Semi | 0 | 0 | 1 | 10 | 10 | 22 | 17 | 40 | 27 | 22 | 10 | 8 | 2 | 6 | 9 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 188 |
| 02Semi | 0 | 0 | 2 | 11 | 14 | 11 | 25 | 27 | 30 | 19 | 9 | 15 | 5 | 3 | 8 | 4 | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 188 |
| 99SqPass | 0 | 1 | 0 | 16 | 16 | 16 | 19 | 38 | 23 | 18 | 9 | 5 | 7 | 2 | 3 | 2 | 2 | 0 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 182 |
| 02SqPass | 0 | 0 | 0 | 11 | 14 | 19 | 17 | 29 | 39 | 21 | 6 | 10 | 5 | 2 | 4 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 184 |
| 99PtGam | 0 | 0 | 4 | 11 | 13 | 22 | 17 | 31 | 24 | 26 | 11 | 10 | 2 | 2 | 8 | 1 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 188 |
| 02PtGam | 0 | 0 | 0 | 6 | 4 | 7 | 16 | 19 | 11 | 9 | 4 | 2 | 2 | 2 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 86 |
| 99Fidalgo | 0 | 0 | 1 | 12 | 10 | 14 | 21 | 39 | 27 | 10 | 13 | 7 | 4 | 5 | 4 | 1 | 3 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 174 |
| 95PrinceW | 0 | 0 | 1 | 10 | 17 | 18 | 27 | 31 | 26 | 21 | 11 | 4 | 3 | 3 | 7 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 184 |
| 96NortonS | 0 | 2 | 2 | 15 | 10 | 17 | 19 | 33 | 38 | 16 | 15 | 7 | 3 | 4 | 8 | 3 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 198 |
| 99Northum | 0 | 0 | 0 | 18 | 10 | 20 | 30 | 36 | 23 | 19 | 7 | 7 | 1 | 1 | 11 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 188 |
| 98SFBay | 1 | 0 | 3 | 10 | 2 | 10 | 8 | 14 | 22 | 11 | 9 | 5 | 4 | 1 | 7 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 112 |
| Total | 1 | 3 | 22 | 179 | 192 | 255 | 319 | 440 | 404 | 309 | 150 | 115 | 53 | 44 | 96 | 25 | 24 | 14 | 9 | 12 | 5 | 9 | 1 | 1 | 5 | 1 | 2688 |

| *Cpa-A* | 182 | 186 | 190 | 194 | 198 | 202 | 206 | 210 | 214 | 218 | 222 | 226 | 230 | 234 | 238 | 242 | 246 | 250 | 254 | 258 | 262 | 266 | 270 | 274 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 0 | 6 | 8 | 6 | 44 | 15 | 6 | 6 | 6 | 6 | 6 | 5 | 4 | 4 | 6 | 0 | 8 | 2 | 3 | 3 | 3 | 1 | 2 |
| 99ChPt | 0 | 0 | 10 | 11 | 7 | 39 | 14 | 4 | 6 | 5 | 6 | 13 | 1 | 5 | 1 | 4 | 1 | 1 | 4 | 5 | 5 | 0 | 2 | 4 |
| 00ChPt | 0 | 0 | 7 | 7 | 1 | 43 | 17 | 4 | 5 | 6 | 1 | 1 | 4 | 4 | 4 | 3 | 3 | 1 | 1 | 4 | 3 | 3 | 0 | 2 |
| 02CHPt | 1 | 0 | 2 | 7 | 4 | 33 | 10 | 4 | 6 | 8 | 7 | 7 | 7 | 2 | 5 | 2 | 4 | 1 | 2 | 3 | 2 | 1 | 1 | 3 |
| 03ChPt | 0 | 0 | 3 | 12 | 9 | 51 | 13 | 1 | 6 | 6 | 1 | 5 | 5 | 4 | 2 | 4 | 1 | 0 | 3 | 5 | 1 | 0 | 1 | 2 |
| 99Semi | 0 | 0 | 6 | 5 | 4 | 32 | 11 | 6 | 5 | 10 | 4 | 9 | 8 | 8 | 4 | 4 | 7 | 2 | 2 | 2 | 1 | 0 | 3 | 3 |
| 02Semi | 0 | 0 | 4 | 3 | 9 | 45 | 16 | 8 | 8 | 9 | 8 | 8 | 5 | 5 | 5 | 0 | 4 | 4 | 4 | 2 | 1 | 0 | 2 | 3 |
| 99SqPass | 1 | 0 | 5 | 7 | 5 | 29 | 16 | 5 | 3 | 4 | 7 | 5 | 0 | 8 | 7 | 2 | 2 | 3 | 4 | 1 | 1 | 0 | 3 | 5 |
| 02SqPass | 0 | 0 | 2 | 10 | 6 | 41 | 14 | 7 | 7 | 6 | 7 | 8 | 6 | 4 | 4 | 5 | 1 | 3 | 0 | 4 | 0 | 4 | 1 | 4 |
| 99PtGam | 0 | 1 | 8 | 14 | 9 | 39 | 12 | 5 | 6 | 6 | 8 | 16 | 6 | 11 | 4 | 2 | 3 | 1 | 2 | 4 | 3 | 2 | 0 | 3 |
| 02PtGam | 0 | 0 | 6 | 5 | 3 | 25 | 14 | 3 | 3 | 10 | 2 | 5 | 0 | 3 | 1 | 3 | 0 | 0 | 1 | 0 | 3 | 1 | 0 | 0 |
| 99Fidalgo | 1 | 2 | 4 | 7 | 6 | 51 | 6 | 1 | 4 | 3 | 4 | 5 | 2 | 2 | 1 | 2 | 2 | 0 | 0 | 8 | 0 | 1 | 1 | 1 |
| 95PrinceW | 0 | 0 | 8 | 10 | 7 | 38 | 13 | 8 | 11 | 5 | 7 | 6 | 6 | 5 | 8 | 0 | 2 | 0 | 1 | 2 | 2 | 2 | 2 | 2 |
| 96NortonS | 0 | 2 | 11 | 9 | 7 | 39 | 17 | 8 | 8 | 8 | 7 | 10 | 3 | 11 | 6 | 2 | 2 | 0 | 1 | 5 | 2 | 3 | 0 | 4 |
| 99Northum | 0 | 0 | 2 | 5 | 7 | 38 | 9 | 4 | 7 | 7 | 8 | 6 | 11 | 10 | 1 | 5 | 6 | 2 | 1 | 4 | 3 | 0 | 4 | 1 |
| 98SFBay | 0 | 2 | 0 | 4 | 4 | 50 | 5 | 6 | 4 | 1 | 2 | 8 | 1 | 4 | 0 | 4 | 0 | 1 | 0 | 1 | 0 | 1 | 2 | 0 |
| Total | 3 | 7 | 84 | 124 | 94 | 637 | 202 | 80 | 95 | 100 | 85 | 118 | 70 | 90 | 57 | 48 | 38 | 27 | 28 | 53 | 30 | 21 | 23 | 39 |

| *Cpa-A* cont. | 278 | 282 | 286 | 290 | 294 | 298 | 302 | 306 | 310 | 314 | 318 | 322 | 326 | 330 | 334 | 338 | 342 | 346 | 350 | 354 | 358 | 362 | 366 | 370 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 3 | 3 | 0 | 2 | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| 99ChPt | 1 | 3 | 2 | 0 | 1 | 1 | 1 | 1 | 2 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 00ChPt | 0 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| 02CHPt | 1 | 1 | 1 | 3 | 1 | 0 | 3 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 2 | 1 | 4 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 03ChPt | 0 | 2 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 99Semi | 3 | 2 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 02Semi | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 1 | 2 | 1 | 2 | 3 | 0 | 1 | 0 | 0 | 0 | 1 |
| 99SqPass | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 2 | 1 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 02SqPass | 1 | 0 | 2 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 5 | 4 | 2 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | 3 | 2 | 1 | 1 |
| 99PtGam | 2 | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 3 | 6 | 1 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 3 | 0 |
| 02PtGam | 0 | 2 | 0 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 99Fidalgo | 2 | 3 | 3 | 1 | 0 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 95PrinceW | 2 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 2 | 4 | 5 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 96NortonS | 0 | 0 | 1 | 2 | 2 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 2 | 2 | 0 | 1 | 3 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| 99Northum | 2 | 1 | 4 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |

| *Cpa-A* cont. | 278 | 282 | 286 | 290 | 294 | 298 | 302 | 306 | 310 | 314 | 318 | 322 | 326 | 330 | 334 | 338 | 342 | 346 | 350 | 354 | 358 | 362 | 366 | 370 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 98SFBay | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| Total | 21 | 18 | 19 | 16 | 11 | 16 | 15 | 11 | 9 | 10 | 10 | 15 | 24 | 7 | 9 | 12 | 15 | 8 | 6 | 2 | 4 | 8 | 9 | 6 |

| *Cpa-A* cont. | 374 | 378 | 382 | 386 | 390 | 394 | 398 | 402 | 406 | 410 | 414 | 418 | 426 | 430 | 442 | 446 | 454 | 458 | 474 | 482 | 490 | 502 | total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 168 |
| 99ChPt | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 168 |
| 00ChPt | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 140 |
| 02CHPt | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 150 |
| 03ChPt | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 148 |
| 99Semi | 2 | 1 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 156 |
| 02Semi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 174 |
| 99SqPass | 2 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 142 |
| 02SqPass | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 178 |
| 99PtGam | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 192 |
| 02PtGam | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 94 |
| 99Fidalgo | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 132 |
| 95PrinceW | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 180 |
| 96NortonS | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 | 0 | 0 | 0 | 196 |
| 99Northum | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 160 |
| 98SFBay | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 116 |
| Total | 8 | 5 | 1 | 6 | 5 | 2 | 6 | 1 | 2 | 5 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 3 | 3 | 1 | 2 | 1 | 2494 |

| *Cpa-H* | 228 | 232 | 236 | 240 | 244 | 248 | 252 | 256 | 260 | 264 | 268 | 272 | 276 | 280 | 284 | 288 | 292 | 296 | 300 | 304 | 308 | 312 | 316 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 1 | 7 | 8 | 16 | 11 | 12 | 4 | 3 | 9 | 11 | 20 | 8 | 14 | 3 | 9 | 28 | 7 | 5 | 3 | 1 | 1 | 1 | 182 |
| 99ChPt | 0 | 0 | 13 | 8 | 27 | 22 | 15 | 3 | 2 | 7 | 15 | 11 | 4 | 5 | 5 | 9 | 14 | 3 | 14 | 0 | 3 | 0 | 0 | 180 |
| 00ChPt | 0 | 0 | 17 | 4 | 26 | 14 | 17 | 7 | 2 | 8 | 12 | 16 | 10 | 5 | 4 | 3 | 15 | 6 | 17 | 0 | 1 | 0 | 0 | 184 |
| 02CHPt | 0 | 0 | 12 | 3 | 16 | 12 | 9 | 6 | 5 | 9 | 13 | 10 | 4 | 3 | 4 | 2 | 10 | 7 | 7 | 1 | 1 | 0 | 0 | 134 |
| 03ChPt | 0 | 0 | 8 | 1 | 22 | 18 | 9 | 6 | 3 | 10 | 12 | 21 | 5 | 9 | 3 | 11 | 13 | 2 | 14 | 5 | 0 | 0 | 0 | 172 |
| 99Semi | 0 | 0 | 8 | 8 | 17 | 26 | 12 | 4 | 4 | 4 | 8 | 14 | 12 | 12 | 7 | 9 | 24 | 8 | 3 | 5 | 1 | 0 | 0 | 186 |
| 02Semi | 0 | 0 | 7 | 9 | 19 | 11 | 15 | 9 | 2 | 6 | 12 | 23 | 5 | 13 | 2 | 9 | 28 | 8 | 6 | 3 | 2 | 0 | 1 | 190 |
| 99SqPass | 1 | 0 | 7 | 6 | 15 | 8 | 13 | 7 | 5 | 10 | 14 | 23 | 5 | 8 | 5 | 12 | 23 | 8 | 5 | 6 | 1 | 0 | 0 | 182 |
| 02SqPass | 1 | 0 | 10 | 6 | 16 | 12 | 11 | 9 | 2 | 4 | 10 | 28 | 7 | 12 | 3 | 2 | 24 | 11 | 7 | 3 | 0 | 1 | 1 | 180 |
| 99PtGam | 0 | 0 | 10 | 11 | 17 | 9 | 10 | 2 | 3 | 7 | 12 | 11 | 9 | 16 | 10 | 11 | 34 | 7 | 6 | 3 | 0 | 0 | 0 | 188 |
| 02PtGam | 0 | 0 | 4 | 1 | 9 | 4 | 6 | 4 | 3 | 1 | 5 | 13 | 3 | 6 | 3 | 3 | 16 | 4 | 2 | 1 | 0 | 0 | 0 | 88 |

| *Cpa-H* cont | 228 | 232 | 236 | 240 | 244 | 248 | 252 | 256 | 260 | 264 | 268 | 272 | 276 | 280 | 284 | 288 | 292 | 296 | 300 | 304 | 308 | 312 | 316 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99Fidalgo | 0 | 1 | 10 | 9 | 16 | 18 | 14 | 8 | 7 | 1 | 11 | 15 | 8 | 10 | 4 | 7 | 20 | 9 | 2 | 2 | 1 | 1 | 0 | 174 |
| 95PrinceW | 0 | 0 | 6 | 4 | 18 | 17 | 4 | 2 | 4 | 9 | 12 | 28 | 14 | 9 | 5 | 15 | 21 | 3 | 4 | 3 | 1 | 0 | 1 | 180 |
| 96NortonS | 0 | 0 | 9 | 9 | 21 | 13 | 10 | 5 | 8 | 9 | 7 | 24 | 12 | 7 | 8 | 11 | 27 | 7 | 6 | 0 | 0 | 0 | 1 | 194 |
| 99Northum | 0 | 0 | 11 | 17 | 17 | 12 | 3 | 9 | 4 | 7 | 8 | 18 | 9 | 13 | 8 | 5 | 32 | 9 | 4 | 1 | 1 | 0 | 0 | 188 |
| 98SFBay | 0 | 0 | 2 | 2 | 8 | 7 | 1 | 0 | 1 | 11 | 11 | 12 | 2 | 16 | 8 | 11 | 6 | 6 | 11 | 1 | 0 | 0 | 0 | 116 |
| Total | 2 | 2 | 141 | 106 | 280 | 214 | 161 | 85 | 58 | 112 | 173 | 287 | 117 | 158 | 82 | 129 | 335 | 105 | 113 | 37 | 13 | 3 | 5 | 2718 |

| *Cpa-172* | 175 | 179 | 183 | 187 | 191 | 195 | 199 | 203 | 207 | 211 | 215 | 219 | 223 | 227 | 231 | 235 | 239 | 243 | 247 | 251 | 255 | 259 | 263 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 0 | 1 | 4 | 3 | 8 | 18 | 22 | 46 | 21 | 14 | 23 | 14 | 2 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 180 |
| 99ChPt | 2 | 1 | 0 | 2 | 9 | 10 | 19 | 17 | 40 | 20 | 9 | 14 | 20 | 6 | 2 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 178 |
| 00ChPt | 3 | 1 | 0 | 1 | 4 | 10 | 7 | 27 | 43 | 17 | 13 | 23 | 16 | 14 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 186 |
| 02CHPt | 0 | 0 | 0 | 1 | 5 | 14 | 14 | 22 | 30 | 18 | 23 | 22 | 13 | 6 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 176 |
| 03ChPt | 1 | 0 | 1 | 1 | 5 | 12 | 13 | 17 | 37 | 15 | 15 | 15 | 16 | 10 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 162 |
| 99Semi | 0 | 0 | 2 | 0 | 3 | 15 | 26 | 24 | 32 | 25 | 18 | 20 | 12 | 3 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 188 |
| 02Semi | 1 | 0 | 0 | 1 | 3 | 14 | 23 | 27 | 30 | 24 | 16 | 26 | 10 | 7 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 188 |
| 99SqPass | 0 | 0 | 4 | 1 | 5 | 18 | 16 | 10 | 31 | 22 | 7 | 13 | 16 | 7 | 1 | 2 | 1 | 4 | 2 | 0 | 0 | 0 | 0 | 160 |
| 02SqPass | 0 | 0 | 2 | 8 | 5 | 18 | 11 | 17 | 36 | 21 | 17 | 18 | 18 | 4 | 0 | 2 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 180 |
| 99PtGam | 0 | 0 | 2 | 1 | 4 | 22 | 11 | 17 | 39 | 22 | 17 | 17 | 18 | 9 | 4 | 5 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 190 |
| 02PtGam | 0 | 0 | 0 | 1 | 4 | 7 | 4 | 5 | 12 | 9 | 9 | 6 | 5 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 66 |
| 99Fidalgo | 0 | 0 | 0 | 0 | 5 | 14 | 12 | 23 | 28 | 23 | 14 | 22 | 11 | 1 | 4 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 160 |
| 95PrinceW | 3 | 0 | 2 | 2 | 3 | 15 | 16 | 26 | 39 | 24 | 12 | 13 | 14 | 4 | 6 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 184 |
| 96NortonS | 2 | 1 | 0 | 1 | 14 | 17 | 8 | 28 | 36 | 31 | 15 | 16 | 14 | 6 | 3 | 5 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 200 |
| 99Northum | 2 | 1 | 1 | 4 | 2 | 23 | 17 | 27 | 32 | 31 | 13 | 14 | 6 | 4 | 2 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 182 |
| 98SFBay | 1 | 0 | 0 | 1 | 4 | 8 | 6 | 17 | 22 | 11 | 7 | 21 | 7 | 8 | 2 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 120 |
| Total | 15 | 4 | 15 | 29 | 78 | 225 | 221 | 326 | 533 | 334 | 219 | 283 | 210 | 94 | 39 | 32 | 13 | 11 | 2 | 3 | 3 | 5 | 6 | 2700 |

| *Cha-6* | 158 | 162 | 166 | 170 | 174 | 178 | 182 | 186 | 190 | 194 | 198 | 202 | 206 | 210 | 214 | 218 | 222 | 226 | 230 | 234 | 238 | 258 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 1 | 0 | 109 | 31 | 6 | 3 | 5 | 9 | 0 | 6 | 4 | 3 | 8 | 1 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 190 |
| 99ChPt | 0 | 0 | 0 | 74 | 24 | 3 | 2 | 6 | 6 | 4 | 5 | 5 | 4 | 4 | 7 | 1 | 5 | 1 | 0 | 1 | 0 | 0 | 152 |
| 00ChPt | 0 | 0 | 1 | 92 | 23 | 2 | 1 | 6 | 4 | 9 | 6 | 6 | 2 | 6 | 10 | 3 | 5 | 0 | 1 | 0 | 1 | 0 | 178 |
| 02CHPt | 0 | 1 | 1 | 69 | 24 | 4 | 4 | 10 | 7 | 6 | 9 | 6 | 10 | 8 | 4 | 2 | 4 | 0 | 1 | 0 | 0 | 0 | 170 |
| 03ChPt | 0 | 0 | 1 | 97 | 18 | 0 | 0 | 16 | 3 | 5 | 3 | 7 | 6 | 5 | 5 | 2 | 2 | 1 | 0 | 0 | 0 | 1 | 172 |
| 99Semi | 0 | 0 | 0 | 86 | 27 | 4 | 1 | 6 | 7 | 3 | 5 | 4 | 1 | 3 | 2 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 154 |
| 02Semi | 0 | 0 | 1 | 88 | 34 | 2 | 2 | 12 | 15 | 5 | 0 | 11 | 7 | 4 | 2 | 1 | 2 | 3 | 0 | 1 | 0 | 0 | 190 |

| *Cha-6* cont | 158 | 162 | 166 | 170 | 174 | 178 | 182 | 186 | 190 | 194 | 198 | 202 | 206 | 210 | 214 | 218 | 222 | 226 | 230 | 234 | 238 | 258 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99SqPass | 0 | 0 | 0 | 104 | 33 | 4 | 1 | 5 | 8 | 4 | 3 | 5 | 2 | 10 | 3 | 4 | 1 | 2 | 0 | 3 | 0 | 0 | 192 |
| 02SqPass | 0 | 0 | 0 | 91 | 25 | 2 | 2 | 9 | 10 | 8 | 5 | 7 | 5 | 7 | 4 | 3 | 2 | 2 | 1 | 1 | 0 | 0 | 184 |
| 99PtGam | 0 | 0 | 1 | 88 | 30 | 2 | 1 | 7 | 6 | 9 | 7 | 10 | 8 | 9 | 1 | 3 | 1 | 0 | 0 | 2 | 0 | 1 | 186 |
| 02PtGam | 0 | 0 | 0 | 53 | 18 | 0 | 1 | 3 | 5 | 5 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 94 |
| 99Fidalgo | 0 | 0 | 1 | 78 | 29 | 3 | 2 | 8 | 13 | 8 | 2 | 8 | 3 | 7 | 3 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 168 |
| 95PrinceW | 0 | 0 | 1 | 75 | 16 | 5 | 2 | 10 | 19 | 9 | 11 | 10 | 5 | 9 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 176 |
| 96NortonS | 1 | 0 | 1 | 94 | 19 | 8 | 2 | 14 | 14 | 8 | 8 | 8 | 4 | 7 | 3 | 3 | 1 | 2 | 0 | 3 | 0 | 0 | 200 |
| 99Northum | 0 | 0 | 2 | 96 | 25 | 5 | 2 | 4 | 6 | 7 | 5 | 7 | 13 | 4 | 5 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 184 |
| 98SFBay | 0 | 0 | 1 | 54 | 25 | 2 | 9 | 2 | 1 | 4 | 4 | 8 | 4 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 118 |
| Total | 1 | 2 | 11 | 1348 | 401 | 52 | 35 | 123 | 133 | 94 | 82 | 109 | 80 | 93 | 52 | 34 | 28 | 11 | 3 | 13 | 1 | 2 | 2708 |

| *Cpa-130* | 204 | 208 | 212 | 216 | 220 | 224 | 228 | 232 | 236 | 240 | 244 | 248 | 252 | 256 | 260 | 264 | 268 | 272 | 276 | 280 | 284 | 288 | 292 | 296 | 300 | 304 | 308 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 99ChPt(ns) | 0 | 21 | 8 | 34 | 65 | 17 | 21 | 9 | 5 | 2 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 186 |
| 99ChPt | 1 | 19 | 3 | 12 | 34 | 13 | 14 | 6 | 4 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 112 |
| 00ChPt | 2 | 19 | 6 | 17 | 65 | 22 | 35 | 9 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 186 |
| 02CHPt | 0 | 18 | 5 | 19 | 49 | 25 | 23 | 7 | 7 | 3 | 2 | 2 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 164 |
| 03ChPt | 1 | 20 | 7 | 20 | 58 | 21 | 26 | 13 | 7 | 0 | 3 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 178 |
| 99Semi | 3 | 23 | 7 | 32 | 51 | 21 | 17 | 2 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 162 |
| 02Semi | 1 | 22 | 10 | 32 | 64 | 18 | 22 | 8 | 9 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 190 |
| 99SqPass | 0 | 15 | 5 | 21 | 46 | 19 | 13 | 13 | 22 | 2 | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 164 |
| 02SqPass | 1 | 12 | 8 | 22 | 47 | 20 | 29 | 14 | 20 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 174 |
| 99PtGam | 0 | 17 | 12 | 33 | 57 | 21 | 20 | 3 | 8 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 178 |
| 02PtGam | 0 | 9 | 2 | 16 | 34 | 5 | 19 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 92 |
| 99Fidalgo | 0 | 35 | 2 | 21 | 46 | 20 | 19 | 8 | 7 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 162 |
| 95PrinceW | 1 | 28 | 6 | 24 | 30 | 34 | 25 | 12 | 8 | 6 | 3 | 2 | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 184 |
| 96NortonS | 0 | 21 | 4 | 22 | 46 | 29 | 34 | 8 | 11 | 3 | 2 | 1 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 186 |
| 99Northum | 0 | 18 | 9 | 30 | 52 | 18 | 31 | 12 | 6 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 180 |
| 98SFBay | 0 | 16 | 34 | 31 | 9 | 10 | 3 | 2 | 1 | 5 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 116 |
| Total | 10 | 313 | 128 | 386 | 753 | 313 | 351 | 131 | 123 | 32 | 19 | 10 | 4 | 3 | 8 | 2 | 5 | 2 | 3 | 2 | 1 | 2 | 1 | 4 | 2 | 1 | 5 | 2614 |

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# Pacific and Atlantic herring produce burst pulse sounds

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**The commercial importance of Pacific and Atlantic herring (*Clupea pallasii* and *Clupea harengus*) has ensured that much of their biology has received attention. However, their sound production remains poorly studied. We describe the sounds made by captive wild-caught herring. Pacific herring produce distinctive bursts of pulses, termed Fast Repetitive Tick (FRT) sounds. These trains of broadband pulses (1.7–22 kHz) lasted between 0.6 s and 7.6 s. Most were produced at night; feeding regime did not affect their frequency, and fish produced FRT sounds without direct access to the air. Digestive gas or gulped air transfer to the swim bladder, therefore, do not appear to be responsible for FRT sound generation. Atlantic herring also produce FRT sounds, and video analysis showed an association with bubble expulsion from the anal duct region (i.e. from the gut or swim bladder). To the best of the authors' knowledge, sound production by such means has not previously been described. The function(s) of these sounds are unknown, but as the *per capita* rates of sound production by fish at higher densities were greater, social mediation appears likely. These sounds may have consequences for our understanding of herring behaviour and the effects of noise pollution.**

**Keywords:** fish; sound production; sound characteristics; marine mammal; swim bladder

## 1. INTRODUCTION

The commercial importance of the clupeoid sub-order, particularly the Pacific and Atlantic herring (*Clupea pallasii* and *Clupea harengus*), has ensured that their biology has received considerable attention (Blaxter 1985). Sound reception in these fishes appears to be unusually well developed, implying that hearing is important to them (Blaxter & Hunter 1982). However, it is unknown which sounds their hearing structures were developed to receive. One possibility is the high-frequency sounds produced by echolocating cetaceans (Mann *et al.* 1997; Wilson & Dill 2002). Another may be sounds made by the fishes themselves.

Little is known about the acoustic emissions of herring. Described sounds fall into three categories. Incidental noises include those associated with jaw movements while feeding, and hydrodynamic sounds from moving schools (Fish & Mowbray 1970). Tonal sounds, termed 'whistles', have been detected in the vicinity of herring at night (Schwarz & Greer 1984). Pulsed sounds vary from thumps to low-frequency pressure pulses (Fish & Mowbray 1970) and, in one study of Pacific herring, include bursts of broadband pulses (Schwarz & Greer 1984).

The production mechanism and function of tonal and broadband pulsed sounds are unknown. Various sonic mechanisms are recognized in fishes but no special adaptations have been identified in herring. Because herring have no gas gland, swim bladder re-inflation is thought to occur by transferral, via the stomach, of gulped surface air (Blaxter & Batty 1984). This transferral has been described as a mechanism with potential for incidental sound production (Fish & Mowbray 1970). Although it is feasible that all herring sound production is incidental, the complexity of the tonal and pulsed sounds, coupled with the species' own hearing abilities, raise the potential for communicative function(s). The ramifications of acoustic communication by these widespread, ecologically and economically important species prompted us specifically to investigate their potential for sound production.

## 2. MATERIAL AND METHODS

Pacific herring (mean fork length: $170 \pm 30$ mm (s.d.), $n = 400$) were caught off Vancouver Island, British Columbia and held at the Bamfield Marine Science Centre. Atlantic herring were caught near Oban and held at the Dunstaffnage Marine Laboratory, Scotland (mean fork length: $208 \pm 12$ mm (s.d.)). Experiments on Pacific herring were carried out in two 500 l tanks. Recordings were made with a calibrated Cetacean Research Technologies C50a omnidirectional hydrophone suspended at mid-depth in each tank and connected to a DAT recorder sampling at 44.1 kHz. In-water light levels were monitored with a LI-COR spherical probe and datalogger (LI-COR LI1000). Measurements of sound pressure levels were carried out in a circular 1.86 m diameter fibreglass tank. The tank was divided with a net twine barrier to keep fishes at least 1 m from the hydrophone. For feeding trials, fishes were either deprived of food for 48 h prior to the trials or fed 28 h and 4 h before, and then every 2 h during, the trials.

To determine whether access to surface air was required for sound production, 20 Pacific herring were placed in each of two tanks. In the 'screened' tank, a rigid mesh screen was fixed below the surface to deny the fishes access to the air–water interface. In the second 'unscreened' tank, the mesh was fixed above the air–water interface. On the night following fish introduction, the number of Fast Repetitive Tick (FRT) sounds in each tank was recorded for 120 min. To examine whether the presence of predatory shark odour impacted sound production, two tanks holding herring were set side-by-side. One was fed water directly from a container holding adult dogfish (*Squalus acanthias*) at high density and the other was fed water from an unoccupied container. To observe herring during sound production, nocturnal video recordings of Atlantic herring were carried out with a camera, infrared floodlights and hydrophone, as described above. Individual fishes were not re-used in any experiments.

## 3. RESULTS AND DISCUSSION

The most distinctive sounds detected during our study were the trains of FRT sounds (see electronic Appendix A, available on The Royal Society's Publications Web site). The best recordings of FRT sounds came from juvenile and adult Pacific herring. FRT sounds consisted of discrete stereotyped bursts of 7–65 pulses (mean of 32, $n = 20$; figure 1) lasting 0.6–7.6 s (mean of 2.6). They


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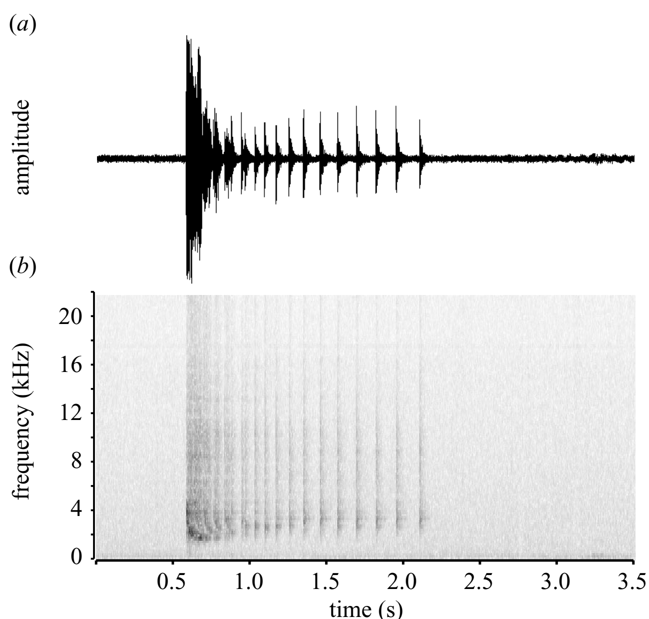


Figure 1. (*a*) Waveform and (*b*) spectrogram of a typical FRT sound. Sampling rate of 44.1 kHz. In (*b*), the fast Fourier transform length is 256, frequency resolution is 86 Hz and time resolution is 11.6 ms.

comprised a single continuous burst train rather than intermittent bursts and, within a train, the time-interval between successive pulses increased in a characteristic fashion. The pulses themselves were broadband with frequencies from 1.7 to at least 22 kHz (this study's frequency ceiling; figure 1). The spectral composition of pulses varied subtly during each FRT sound, notably in the first few pulses (figure 1). The rapid succession of pulses, smooth transition of interpulse intervals and absence of overlapping or out-of-phase pulse trains suggest that each FRT sound was produced by an individual fish. The presence of high-frequency components and absence of low frequencies makes these sounds unusual when compared with other fish sounds (Fish & Mowbray 1970). Precise measurement of the amplitude of the FRT sounds proved problematic, but from a sample of 13, a conservative measurement of sound pressure level of 143 dB re1µPa @ 1–1.8 m (peak) was recorded.

Herring at sea typically follow a diel behavioural rhythm, forming diurnal deep-water schools and nocturnal loose surface shoals (Blaxter & Parrish 1965). If some component of the process of transferring gulped surface air to the swim bladder generates the FRT sounds (Fish & Mowbray 1970) then we might expect that these sounds would be most prevalent at night. This was found to be the case. Sounds made by eight groups of 20 Pacific herring were sampled over 24 h periods. The diel distribution of FRT sounds differed significantly from random (see figure 2; $p < 0.001$) with most occurring after dark (23.00 to midnight PST). During this experiment, fed and unfed fishes produced similar numbers of FRT sounds (fed: 10; food deprived: 13).

To investigate the transfer of gulped air to swim bladder hypothesis further, we tested whether access to surface air was required for FRT sound production, using the screened and unscreened tanks described above. FRT sounds were recorded in both tanks (mean screened: 6; unscreened: 9; $n = 4$ replicate trials). We therefore conclude that access to surface air is not immediately necessary for FRT sound production. For two out of the four trials, monitoring was continued for a second and third night after fish introduction. The number of FRT sounds recorded on these nights in the screened tank was much lower (mean of 0.75), whereas in the unscreened tank they remained as numerous (mean of 9). Thus, although access to the air–water interface is not immediately necessary for FRT sound production, previous air access does appear important.

Atlantic herring also produce FRT sounds. Simultaneous nocturnal acoustic and infrared video recordings of 50 captive juvenile Atlantic herring showed that FRT sounds were temporally associated with the appearance of fine bubble streams from the anus or anal duct of individual fishes (see electronic Appendix A). The fish that produced the bubbles appeared otherwise normal during bubble emission.

It is unlikely that FRT sound generation results from swim bladder inflation with gulped or digestive gases or from buoyancy trimming subsequent to inflation. We reached this conclusion following observations that bubbles were vented from the anal duct simultaneously with the occurrence of FRT sounds and that fish denied access to the surface or deprived of food produced these sounds. Instead, we suggest that FRT sound production is associated with gas expulsion from the swim bladder via the anal duct, a form of sound production not, to our knowledge, previously described in fishes. This notion appears to be supported by the reduction in FRT sounds by fishes that have been denied access to the surface for more than one night. This is because these fishes are likely to have under-inflated swim bladders owing to previous FRT production or diffusion of gas from the swim bladder (Blaxter & Batty 1984). Although herring release gas through the anal duct in response to rapid pressure reductions (Brawn 1962), it is unclear why captive fishes in shallow tanks, other than through habit, should vent gas nocturnally to control their buoyancy. Gas venting and associated sounds might instead have other function(s).

The capacity of herring to respond to various underwater sounds is well documented (Schwarz & Greer 1984; Wilson & Dill 2002). Early studies of clupeoids indicated that their auditory range extended to 1 kHz, but more recent work has shown that they can detect sounds at higher, and in some instances considerably higher, frequencies (Mann *et al.* 2001). It is conceivable, therefore, that they can detect FRT sounds made by conspecifics, creating the potential for intraspecific communication. FRT sounds could function in mate location/choice or as alarm calls, but these possibilities are unlikely since the fishes in this study were not in breeding condition, and FRT sounds were not recorded during disturbance for tank maintenance or upon addition of predatory shark odour. Contact calls are more plausible. It is unknown how herring shoal in darkness, when their schools become scattered and less organized, but visual and olfactory cues are unlikely (Blaxter & Parrish 1965). Acoustic communication could allow fishes to maintain contact. Indeed, when different numbers of Pacific herring (1, 5, 10 and

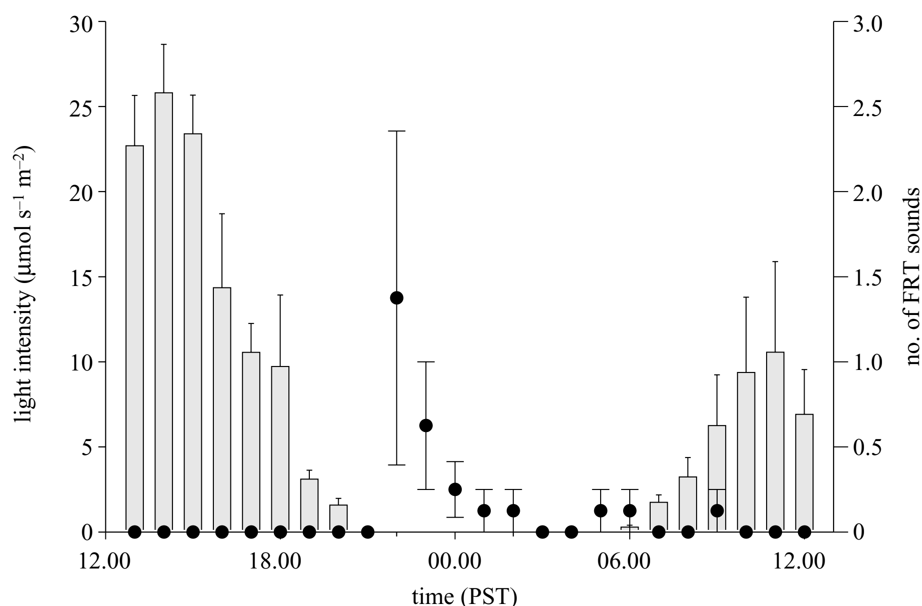


Figure 2. The occurrence of FRT sounds (solid circles) and in-tank light levels (columns). Means ± 1 s.e. The timing of FRT sounds differed significantly from random (Rayleigh test, $r = 0.85$, $p < 0.001$, $n = 8$; Batschelet 1981) during 24 h recordings in July and August 2000. The bulk of FRT sounds occurred after dark, between 23.00 and midnight PST. Recordings were made for 6 minutes every hour. No relationships were found between the occurrence of FRT sounds and oxygen saturation, temperature or salinity ($p = 0.74$, $p = 0.38$, $p = 0.60$, respectively).

30) were held overnight in 500 l tanks, the number of FRT sounds recorded increased disproportionately to the number of fishes present (mean hourly emissions *per capita*: lone fish = 0; 5 fishes = 0.02; 10 fishes = 0.12; 30 fishes = 0.17, OLS regression, $r^2 = 0.46$, $p < 0.001$, $n = 4$ per density treatment), implying that sound production is socially mediated. Calls advertising location would only be advantageous, however, if predators are unable to detect the caller. The unusual frequency structure of the FRT sounds, with most of the energy above 2 kHz, means that they are near or above the known auditory range of most predatory fishes (Fay & Simmons 1999). However, we note that they are well within the detection capabilities of marine mammals.

If herring use sounds to communicate, our understanding of the impacts of anthropogenic noise may require some re-evaluation. Regardless of function, the superficial resemblance of these sounds to those of other marine organisms may necessitate methodological modifications to ensure that herring sounds are not erroneously included in such datasets. By contrast, autonomous acoustic equipment developed to monitor odontocete occurrence (Culik *et al.* 2001) could be adapted to study the presence and abundance of herring. Finally, herring are a major dietary component of many Northern Hemisphere pinniped and cetacean species. Despite considerable efforts to understand how these predators locate prey at sea, our knowledge remains rudimentary. Given the auditory capabilities of these predators, it is conceivable that they use the distinctive herring sounds as foraging cues. Recognition of this cue could give new insight into marine mammal foraging tactics and the impacts of anthropogenic sound pollution on foraging efficiency.

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47. Consensus cladogram of most parsimonious trees for analysis of 173 living taxa of seed plants, plus the fossil *Archaefructus*. Various analyses included 1628 molecular characters and 17 to 108 morphological characters. The molecular characters are based on the three-gene matrix (*rbcL*, *atpB*, *18s*) that was recently published for 567 species (*2*). Taxa were selected to provide a good representation of variation throughout the angiosperms, including a dense sampling of the so-called basal angiosperms. The tree shown was generated with a matrix of 1645 characters (17 morphological characters, including only those relevant characters that could be scored for the fossil). Parsimony analysis was undertaken using the parsimony ratchet of Nixon (*46*), with numerous runs of 200 replications for each analysis. In all analyses, *Archaefructus* is a sister taxon to the angiosperms as shown in this tree. Depending on the data set used, the overall length of the tree varied, with an overall consistency index of ~0.18 (consistent with the original three-gene analysis). The taxa *Cycas*, *Bowenia*, *Zamia*, *Ginkgo*, *Ephedra*, and *Pinus* represent the modern gymnosperms; the other taxa in the analysis are angiosperms (flowering plants). Note that this data set does not address the question of whether the gymnosperms are monophyletic, because no taxa outside of the seed plants were included. The tree has been drawn to be neutral on this point, and it supports either hypothesis equally. The tree differs from the original three-gene analysis only in the position of *Ephedra*, which in these trees is more consistent with analyses of other genes that place gnetopsids with Pinaceae, suggesting that the morphology may play a positive role in resolving discrepancies.
48. See supplemental material on *Science* Online (www.sciencemag.org/cgi/content/full/296/5569/899/DC1).
49. Supported by the National Natural Science Foundation of China (projects 39970050 and 39770059), Key Project of Ministry of Land and Resources, China, Special Project Stz. 98-1-02 (Chinese Academy of Sciences), and by NSF grants INT-0074295 and DEB 0090283 and the Becker/Dilcher Endowment for Paleobotany of the University of Florida Foundation. We thank T. A. Lott for assistance with photography, illustration, and manuscript preparation; K. Simons for diagramming the reconstruction of *Archaefructus*; and J. Y. Liu and C. L. Gao of the Natural Museum of Dalian for their help in observation of some specimens of *Archaefructus*. This paper is University of Florida Contribution to Paleobiology publication number 525.

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# Mammal Population Losses and the Extinction Crisis

**Gerardo Ceballos[1] and Paul R. Ehrlich[2]**

**The disappearance of populations is a prelude to species extinction. No geographically explicit estimates have been made of current population losses of major indicator taxa. Here we compare historic and present distributions of 173 declining mammal species from six continents. These species have collectively lost over 50% of their historic range area, mostly where human activities are intensive. This implies a serious loss of ecosystem services and goods. It also signals a substantial threat to species diversity.**

Population extinctions are a more sensitive indicator of the loss of biological capital than species extinctions. This is because many of the species that have lost a substantial portion of their populations [thus altering ecosystems and perhaps reducing the ability of those systems to deliver services (*1*)] are unlikely to go globally extinct and enter the species extinction statistics in the foreseeable future (*2*). Most analyses of the current loss of biodiversity emphasize species extinctions (*3–5*) and patterns of species decline (*6–8*) and do not convey the true extent of the depletion of humanity's natural capital. To measure that depletion, we need to analyze extinctions of both populations and species. Here we give a rough minimum estimate of the global loss of continental mammal populations. We believe that mammals, because of their great taxonomic diversity and the wide range of ecological niches they exploit, can serve as an indicator of what is occurring in the rest of Earth's biota.

Our data consist of historic (i.e., mostly 19th century) and present-day distributional ranges of all of the terrestrial mammals of Australia and subsets of the terrestrial mammal faunas of Africa, South East Asia, Europe, and North and South America (Table 1 and table S1). These subsets consist of all mammal species whose ranges are known to be shrinking for which we had access to data. They comprise roughly 4% of the ~4650 known species. We assume that loss of range area is due to the extinction of populations, but we do not attempt to equate a given areal loss with a precise number of population extinctions due to the complexities of defining and delimiting populations (*9*). Data were gathered from the specialized literature (Web references). In general, because they are better known, most of our range data are from medium- and large-sized species. Whether globally these are more or less liable to population extinction than medium to small species is a matter of conjecture (*10–12*), but at present there is little reason to assume an important directional bias in our samples. There was no correlation between body mass and range shrinkage in our data ($P > 0.05$, $r^2 = 0.22$). There does remain a possible source of bias in the relative lack of very small species in the total sample (*12*).

The ranges were digitized and the historic and present range areas were calculated. For each species, we estimated both total area occupied historically and percent historic range area now occupied. Using ArcView 3.1, the ranges were superimposed to produce synthetic maps summarizing the losses of species populations in 2 degree by 2 degree quadrats (i.e., the number of species that have disappeared from each quadrat because all of their populations previously located in that quadrat have disappeared). The area of these quadrats, of course, varies with latitude, but the average of such quadrats over land is about 30,000 km$^2$.

Declining species of mammals in our sample had lost from 3 to 100% of their

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geographic ranges (mean 68 ± SE 2.46), but range lost was above 50% for most (72%) species (Table 1). Species such as Pere David's deer (*Elaphurus davidianus*), which is extinct in the wild, lost 100%, whereas others like Spotted hyena (*Crocuta crocuta*) that have a higher tolerance for human disturbance lost 14%. As expected, there were striking differences between the continents, as shown in Table 1 and Figure 1. The number of populations lost has been greater in areas that are both large and species rich (e.g., Africa and Southeast Asia).

In our analysis, population extinctions today seem to be concentrated either where there are high human population densities, or where other human impacts, such as intensive agriculture, grazing, and hunting, have been severe. Larger mammals are often hunted to extinction or have their habitats preempted (*13*, *14*). The mammal faunal sample from Southeast Asia shows one of the highest losses of species ranges and, thus, of mammal population extinctions: 57% of its quadrats have lost between 75 and 100% of their mammals. In Southeast Asia, human population density is extremely high (e.g., Indonesia, 115 persons per km$^2$; China, 130 persons/km$^2$; Pakistan, 190 persons/km$^2$; India, 305 persons/km$^2$). Similarly, in North America, the highest percentage losses are in the heavily populated eastern United States.

In Africa, the areas with the highest levels of mammal population extinction do not coincide as well with high human population densities (e.g., Nigeria has 135 persons/km$^2$), even though there is a positive correlation of human population density with species richness in general (*15*). Rather, the highest percentage of population extinctions have occurred in the region of the Sahara (Mali, 4 persons/km$^2$; Mauritania, 1.5 persons/km$^2$), presumably because gazelles and other large herbivores have been hunted to extinction by local people and sport hunters and because of anthropogenic desertification and competition with domestic animals for scarce forage and water (*16*). In recent years, many populations of tropical species such as gorillas (*Gorilla gorilla*) and drills (*Mandrillus leucophaeus*) have been lost in equatorial Africa (e.g., Congo, where there are 20 persons/km$^2$) (*17*, *18*), but there are no good data on their present geographic ranges. In southern Africa, not surprisingly, the absolute number of extinctions coincides with high population densities of *Homo sapiens*.

Understandably, Australia, which is the continent with the largest number of mammal species extinctions (*12*, *19*), is also a continent showing a widespread severe reduction of populations. Factors causing population and species extinctions there are mainly related to overgrazing, agriculture, forestry practices (including altered fire regimes) (*20*), and, especially, the large numbers of introduced predators and competitors (*21*–*24*).

In South America, population losses are heaviest in the intensively agricultural southern plains (Pampas region in Argentina),

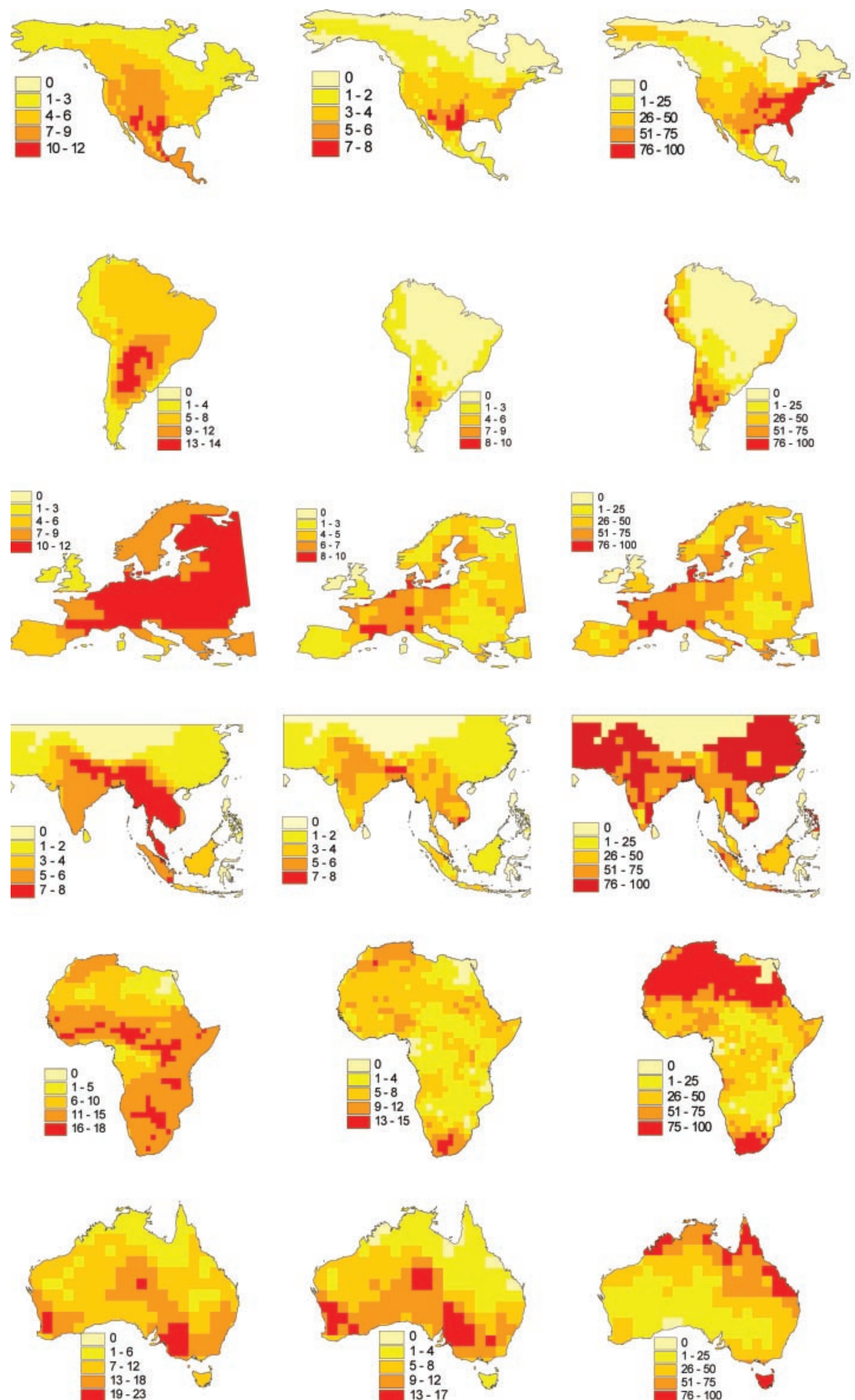


**Fig. 1.** Historic number of species with populations in each 2 degree by 2 degree quadrat (left column of maps), number of species lost from each quadrat (center column), and percentage of species that have disappeared from each quadrat (that is, percentage of population loss) (right column). All data (top to bottom) from species with shrinking ranges in North America (18 spp.), South America (17 spp.), Europe (15 spp.), Southeast Asia (13 spp.; white quadrats at top, outside of range sampled), Africa (52 spp.), and Australia (58 spp).

Mata Atlantica in Brazil, and coastal Ecuador and Peru. Those areas have been devastated by cattle grazing and unsustainable cropping, and they are among the most degraded of that continent (*25*, *26*). In Europe, no distinct pattern emerges even though the continent has been subject to extensive and severe human alteration. One possible reason is that it is a peripheral region with a depauperate mammal fauna that, by the 19th century, may already have lost most species that would decline in the face of anthropogenic disturbance. For example, the wolf (*Canis lupus*), brown bear (*Ursus arctos*), beaver (*Castor fiber*), and other species had been exterminated in Britain by 1700 (*27*, *28*). Therefore, those species were not included in our historic maps of Britain.

In our sample, declining mammal species have collectively lost over 50% of their continental populations (as judged by area loss). If the proportion of declining species in Australia (22%) is typical of the other continents, this would suggest a loss of more than 10% of all mammal populations. But the Australian proportion of decline may be higher than that of other continents. If we make the conservative assumption that the only declining species globally were those in our sample (4% of the global fauna), a loss of about 2% of all mammal populations would still be suggested. Even this is higher than the estimated 1.8% (83 spp.) of global species extinction in Earth's mammal fauna (even though the areas lost in species extinctions have not been estimated and included in population losses), about double the proportion of continental mammal species that have disappeared (less than 1%) (*5*).

Our estimates of population extinctions are necessarily crude. In addition, there are probably two major sources of conservative bias in our study, almost certainly leading to the substantial underestimation of those extinctions. First, even when the distribution of a charismatic endangered species is mapped, the existence of the species in some parts of its "present range" remains doubtful, as in the case of the tiger (*Panthera tigris*) [(*13*) and references therein; J. Ranganathan, personal communication]. We suspect that many less-prominent species, underrepresented in our sample, have lost portions of their ranges but without detection because they have not been subject to intensive mapping attempts.

The second probable conservative bias is potentially even greater. Distribution maps of historic ranges necessarily neglect the many smaller gaps in the distribution representing areas of unsuitable habitat (to take an obvious case, lakes and rivers do not ordinarily appear as blanks in the middle of prairie dog distributions). But we can be sure that anthropogenic habitat alteration has generally created much bigger gaps in the continuous maps that represent present distributions. For example, the map in the standard butterfly guide (*29*) shows the intensely studied *Euphydryas editha* as still occupying almost all of California except the Central Valley. In reality, population extinctions in historic times have removed it from many, if not most, of the sites where it occurred previously (*30*). Similarly, several species such as the monkeys *Leontopithecus rosalia* and *Brachyteles arachnoids* in the Mata Atlantica or the marsupials *Phascogale calura* and *Sminthopsis longicaudata* in Australia have had their historic ranges reduced to tiny fragments of habitat (*12*, *19*, *25*). Nonetheless, they are shown in our present maps as occupying entire quadrats, even though the vast majority of the populations in those quadrats have already gone extinct. If such smaller scale but nearly ubiquitous differences between historic and present mammal distributions could be calculated, losses of area and populations would be much greater.

There is a need to determine more precisely the proportion of mammal species that are shrinking on continents other than Australia, the one continent that has been relatively thoroughly studied, and to investigate the relation of vulnerability to population extinction with respect to body size and other variables on those continents. Also, studies of the details of "range filling" in mammals and other organisms will be critical to measuring more accurately the magnitude of population extinctions. An especially difficult problem is to translate between loss of range area and extinction of populations (*9*).

By definition, conserving population diversity means spreading conservation efforts over wider regions as a complement to important efforts to preserve "hotspots" of species richness (*31*, *32*). Such a regional approach will be made more difficult by the problem of what we call "political endemism," the limitation through population extinctions of a species' geographic range to one or a few political entities. In some cases, if such political entities are not as interested (or capable) in conservation as other entities in the historic range, that may ensure eventual extinction (*33*). A combination of political endemism and political instability has certainly made the fates of the black (*Diceros bicornis*) and Sumatran (*Dicerorhinus sumatrensis*) rhinos much more uncertain (*34*). In both of these conservation cases, a high priority would be to reestablish populations not only over a broader geographic range, but also within a greater variety of countries.

The loss of species diversity has correctly attracted much attention from the general public and decision-makers. It is now the job of the community of environmental scientists to give equal prominence to the issue of the loss of population diversity.

**Table 1.** Average area losses in mammals whose ranges have contracted. Samples were taken from six continents. Asterisk indicates value is from raw data, not from columns to the left.

| Continent | No. of species | Historic range (km²/1000) | Present range (km²/1000) | Range lost (km²/1000) | % Range lost* |
|---|---|---|---|---|---|
| Africa | 52 | 5750 | 2046 | 3704 | 72 |
| North America | 18 | 4735 | 2761 | 1974 | 44 |
| South America | 17 | 5467 | 4648 | 819 | 15 |
| Southeast Asia | 13 | 2677 | 384 | 2293 | 83 |
| Australia | 58 | 1006 | 252 | 754 | 78 |
| Europe | 15 | 3628 | 1122 | 2506 | 72 |
| Total | 173 | | | | |
| Grand mean | | 3599 | 1569 | 2030 | 68 |

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**Supporting Online Material**
(www.sciencemag.org/cgi/content/full/296/5569/904/DC1) table S1
References and notes

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# Genomewide Analysis of mRNA Processing in Yeast Using Splicing-Specific Microarrays

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**Introns interrupt almost every eukaryotic protein-coding gene, yet how the splicing apparatus interprets the genome during messenger RNA (mRNA) synthesis is poorly understood. We designed microarrays to distinguish spliced from unspliced RNA for each intron-containing yeast gene and measured genomewide effects on splicing caused by loss of 18 different mRNA processing factors. After accommodating changes in transcription and decay by using gene-specific indexes, functional relationships between mRNA processing factors can be identified through their common effects on spliced and unspliced RNA. Groups of genes with different dependencies on mRNA processing factors are also apparent. Quantitative polymerase chain reactions confirm the array-based finding that Prp17p and Prp18p are not dispensable for removal of introns with short branchpoint-to-3′ splice site distances.**

Protein-coding information in eukaryotic genomes is fragmented into exons, which must be recognized and joined by the process of RNA splicing. Splicing takes place in the nucleus within a dynamic ribonucleoprotein complex called the spliceosome (*1*). The spliceosome transforms information within transcripts of the eukaryotic genome to create sequences not found in DNA. By its nature and position in the gene expression pathway, splicing expands the possible interpretations of genomic information and does so under developmental and environmental influence (*2*). Our understanding of the process of splicing is derived from studies on relatively few introns. As eukaryotic genomes are sequenced, it has become necessary to ask how the process of splicing is integrated into genome function and evolution. Compared with higher eukaryotes, yeast contains relatively few spliceosomal introns, and most have been correctly annotated (*3*, *4*). Hence, we chose to perform genomewide study of splicing in the yeast *Saccharomyces cerevisiae*.

To discriminate between spliced and unspliced RNAs for each intron-containing yeast gene, we used DNA microarrays (*5*, *6*). Oligonucleotides were designed to detect the splice junction (specific to spliced RNA and not found in the genome), the intron (present in unspliced RNA), and the second exon (common to spliced and unspliced RNA) for each intron-containing gene as shown in Figure 1A. The oligonucleotides were printed on glass slides to create splicing-sensitive microarrays for yeast (*7*).

To determine whether oligonucleotide arrays can function as genomewide sensors of splicing, we compared RNA of cells carrying the temperature-sensitive splicing mutation *prp4-1* with RNA of wild type during a shift from 26°C to 37°C (*7*). Prp4p is an integral component of the spliceosome (*8*, *9*). Plots of fluorescence (*10*) for each oligonucleotide for the wild-type (Cy3) versus the *prp4-1* mutant (Cy5) with time are shown in Fig. 1B. Even at the permissive temperature of 26°C, many intron probes (red spots) display Cy5/Cy3 ratios >1, indicating accumulation of intron-containing RNA in the mutant strain. After the shift to the restrictive temperature, the Cy5/Cy3 ratio increases for most intron probes. In contrast, the ratio decreases for many splice junction probes (green spots), a sign that spliced RNAs become depleted in the mutant. The Cy5/Cy3 ratios for about a thousand intronless genes remain largely unaffected (yellow spots). This indicates that the array reports catastrophic splicing defects and can measure the kinetics of splicing inhibition genomewide.

Despite their conservation, numerous mRNA processing factors are not essential in yeast. To analyze more subtle changes in splicing, we studied 18 mutant strains lacking nonessential genes implicated in mRNA processing (Table 1). Plots of mutant versus wild-type fluorescence intensities for *prp18*Δ, *cus2*Δ, and *dbr1*Δ are shown in Fig. 1C. The effect of each deletion on spliced and unspliced RNA is different. Most severe is *prp18*Δ, which causes widespread intron accumulation and loss of splice junction sequences relative to wild type (Fig. 1C, left). The *cus2*Δ mutation enhances defects in U2 small nuclear RNA (snRNA) or Prp5p (*11*, *12*) but causes little intron accumulation (Fig. 1C, center). Although not required for splicing, Dbr1p debranches the lariat, and its loss results in the dramatic accumulation of intron lariats (*13*). In the *dbr1*Δ strain, most introns accumulate, and there is little effect on spliced mRNAs (Fig. 1C, right). This demonstrates that qualitative differences in splicing phenotype can be distinguished by using splicing sensitive microarrays.

Changes in spliced and unspliced RNA levels due to loss of an mRNA processing factor may arise directly from splicing inhibition or may be due to secondary events that alter transcription or RNA decay. For example, signal from a splice junction probe may increase for a gene whose transcription is induced, even though splicing is inhibited. To

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