PACIFIC SALMON COMMISSION
JOINT CHUM SALMON TECHNICAL COMMITTEE REPORT

TCCHUM (92)-2

ACCURACY AND PRECISION OF GENETIC STOCK IDENTIFICATION FOR ESTIMATING THE STOCK COMPOSITION OF MIXED-STOCK CHUM SALMON FISHERIES IN NORTHERN PUGET SOUND AND SOUTHERN GEORGIA STRAIT

FEBRUARY 18, 1992

Membership of the Chum Technical Committee's GSI Workgroup

Canadian Members	United States Members
Wilf H. Luedke, CDFO	Robert H. Conrad, NWIFC
Dr. Terry Beacham, CDFO	Stevan R. Phelps, WDF
	Dr. Gary Winans, NMFS

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INTRODUCTION

The Chum Technical Committee annually estimates the number of chum salmon of Washington State and British Columbia origin intercepted by commercial salmon fisheries in northern Puget Sound and southern British Columbia. Genetic stock identification (GSI) based on protein electrophoresis is used to estimate the proportion of chum salmon from each country of origin in catches by major intercepting fisheries. The methods used by the two agencies conducting these analyses, the Canadian Department of Fisheries and Oceans (CDFO) and the Washington Department of Fisheries (WDF), are different. Therefore, it is important that the accuracy and precision of the estimates produced by each method are quantified, and any biases identified, so that stock composition estimates can be properly interpreted (Shaklee and Phelps 1990) and correctly applied to catch numbers.

This report summarizes simulations conducted by WDF and CDFO to: (1) quantify the accuracy and precision of the WDF 21-locus, 36-stock baseline (1989 version) used to estimate the stock composition of mixed-stock catches of chum salmon from northern Puget Sound (WDF Statistical Areas 7 and 7A); (2) examine the effect of size of the mixture sample on the accuracy and precision of these estimates; and (3) compare the accuracy and precision of estimates based on 7-loci and 21-loci baselines. Accuracy and precision were quantified using simulations to estimate the stock composition of samples of known stock mixtures (Phelps 1989[1]).

METHODS

Three different models were used for the simulations reported. Model refers to a combination of the treatment of the baseline electrophoretic data, the number of loci analyzed, the analytical method used to estimate stock composition, and the bootstrapping procedures used to estimate standard errors. The three models were: (1) a WDF model using 21 loci; (2) a CDFO model using 21 loci; and (3) a CDFO model using 7 loci (see Appendix Table 1). All three models used the 36-stock baseline (1989 version) used by WDF to estimate the stock composition of chum salmon catches in northern Puget Sound with either all 21 or a subset of 7 loci. Names of the chum salmon stocks in the baseline are listed in Phelps et al. (1991). Dendograms for the 36 stocks in the baseline are shown in Appendix Figures 1a and 1b. Loci used for the WDF and CDFO 21-loci analyses are described in Phelps et al. (1991). Loci used for the CDFO 7-loci analyses are described in Hop Wo et al. (1989). WDF conducted their simulations using their GSI analysis and simulation program (SIMLE). CDFO conducted simulations with their GSI analysis program (Mixture Model 3.11).

[1] "Proposed computer simulations of the WDF GSI chum baseline." Memo from Steve Phelps (Washington Dept. of Fisheries, Olympia WA) to the Chum Technical Committee of the Pacific Salmon Commission. Sept. 26, 1989. 5 p.

Samples of known stock composition were constructed and analyzed to evaluate the accuracy and precision of the three models. All three models estimated the contribution of individual stocks. The individual stock estimates were then pooled to estimate the contribution of the management groups of interest (allocate-sum procedure, Wood et al. 1987). The major differences between the models are described below.

Estimation Procedures

Both agencies use maximum likelihood estimation (MLE) procedures to estimate the stock composition of samples of mixed-stock origin. Although the exact MLE procedures used are different they produce virtually identical estimates when using identical baseline data, convergence criteria, numerical accuracy, and mixture samples (Craig Busack, WDF, Olympia WA, pers. comm.). The two agencies do not use identical baseline data, however. The WDF model uses baseline allele frequencies to generate expected genotype frequencies in Hardy-Weinberg equilibrium prior to analysis while the CDFO model uses observed genotype frequencies from the baselines.

The primary sets of simulations were conducted with a sample size of 200 fish for the simulated mixed-stock samples. Additionally, two sets of simulations were conducted with different mixed-stock sample sizes to examine the effect of sample size on accuracy and precision of the stock composition estimates.

Stock composition estimates reported for the simulations are the mean of 25 bootstrap replications for WDF while CDFO estimates are the mean of 50 bootstrap replications. Standard errors reported by both agencies are the empirical standard deviation of the bootstrap estimates.

Construction of Samples of Known Stock Composition

Although both agencies construct the samples of known stock composition by sampling from the baseline, their exact implementation is different. The WDF simulation program randomly selects a "fish" from a multi-locus genotype array based on the allele frequencies of the baseline stock being simulated. The number of fish sampled from each baseline stock used in the simulated mixed-stock fishery sample is a random sample of the baseline stock proportions. Let X% equal the desired percentage of a stock in a mixture sample being simulated. For the WDF procedure, the mean percentage for a stock in the 25 samples selected during the bootstrap replications tends toward the X% specified for a stock in a mixture sample. For the CDFO simulation procedure, the exact percentage (X%) specified for each stock is in the mixture sample created for each bootstrap replication. Therefore, the estimates from the WDF model incorporate an additional component of variance not present in the CDFO model.

Composition of Samples Used in Simulations

Simulations were conducted with samples of known stock composition representing mixed-stock fishery samples. These samples had varying contributions of Washington (US) and Canadian stocks to examine the accuracy and precision of the estimates from the three models over a range of hypothetical stock compositions. Results were summarized by examining the estimates for three major stock groups; US stocks combined, Fraser River stocks combined, and Georgia Strait stocks combined. For each simulation, the estimated percentage composition of each stock group, the standard error of the estimate, and bias of the estimate (bias = estimated percentage - true percentage) are reported. Mean bias of each set of simulations was used to assess the accuracy of the models and mean standard error of the estimates used to assess the precision of the models. Results were summarized graphically and in tables.

Simulation Set #1:

For the first set of simulations, the ratio of Canadian to US stock groups was varied while the relative contribution of stocks within each region of origin (Canada or US) was held constant. The relative contribution of stocks within each stock group was approximately proportional to estimated stock size. Twelve different percentage contributions of the US stock group were examined by gradually increasing the contribution of US stocks from 0% to 100% (Table 1). The ratio of the three Puget Sound stocks (South Sound, Hood Canal, and North Sound) used to construct the US stock group was kept approximately constant at 1:1:1. Canadian stocks composed the remainder of the samples. The contribution of the Georgia Strait stock group relative to the Fraser River stock group was kept constant at approximately 2:1 (Table 1).

Simulation Set #2:

For the second set of simulations, the stocks contributing to the US stock group were varied. For these simulations, the contribution of the US stock group was held constant at 4% but the stocks contributing to the 4% mixture varied. In one simulation the US stock group was composed of about an equal mixture of the three US stocks (South Sound, Hood Canal, North Sound). For the other three simulations, the US stock group was composed entirely of one of the three Puget Sound stocks (Table 2). The contributions of the Fraser River and Georgia Strait stock groups were held constant at 36% and 60%, respectively (Table 2).

Simulation Set #3:

For the third set of simulations, the contribution of the Fraser River stock group was varied while the total US and Canadian contribution remained constant. The contribution of the US stock group was held constant at 4% and was composed of 1% South Sound, 1% Hood Canal, and 2% North Sound stocks (Table 3). The contribution of the Fraser River stock group to the mixture sample was varied from 0% to 96% (Table 3).

Table 1. Composition of mixture samples used for the first set of simulations (simulation set #1).

Simulation Number	South Sound	Hood Canal	North Sound	Total US	Fraser River	Georgia Strait	Total Canada
1	0%	0%	0%	0%	38%	62%	100%
2	0%	1%	1%	2%	37%	61%	98%
3	1%	1%	2%	4%	36%	60%	96%
4	1%	2%	3%	6%	35%	59%	94%
5	2%	3%	3%	8%	34%	58%	92%
6	3%	3%	4%	10%	33%	57%	90%
7	5%	5%	5%	15%	31%	54%	85%
8	6%	8%	6%	20%	29%	51%	80%
9	14%	13%	13%	40%	21%	39%	60%
10	20%	20%	20%	60%	14%	26%	40%
11	28%	26%	26%	80%	7%	13%	20%
12	34%	33%	33%	100%	0%	0%	0%

Table 2. Composition of mixture samples used for the second set of simulations (simulation set #2).

Simulation Number	South Sound	Hood Canal	North Sound	Total US	Fraser River	Georgia Strait	Total Canada
13	1%	1%	2%	4%	36%	60%	96%
14	4%	0%	0%	4%	36%	60%	96%
15	0%	4%	0%	4%	36%	60%	96%
16	0%	0%	4%	4%	36%	60%	96%

Table 3. Composition of mixture samples used for the third set of simulations (simulation set #3).

Simulation Number	South Sound	Hood Canal	North Sound	Total US	Fraser River	Georgia Strait	Total Canada
17	1%	1%	2%	4%	0%	96%	96%
18	1%	1%	2%	4%	19%	77%	96%
19	1%	1%	2%	4%	36%	60%	96%
20	1%	1%	2%	4%	48%	48%	96%
21	1%	1%	2%	4%	77%	19%	96%
22	1%	1%	2%	4%	96%	0%	96%

Effect of Size of the Mixture Sample

Two sets of simulations examined the effect of the size of the mixture sample on the accuracy and precision of stock composition estimates by each model. The stock group mixtures of the samples used in these simulations were: (1) 0% US:38% Fraser River: 62% Georgia Strait; and (2) 6% US:35% Fraser River:59% Georgia Strait. WDF examined mixture sample sizes of 200 and 400. CDFO examined sample sizes of 100, 200, 300, 400, and 600 for both the 7-loci and 21-loci models.

Comparison of CDFO Models Using 7-loci and 21-loci Baselines

The two CDFO models are identical except one uses 7 loci and the other 21 loci. The effect of the increased number of loci in the baseline on the accuracy and precision of the estimates was examined by comparing the accuracy and precision of these two models. Only estimates for the US and total Canadian (Fraser River and Georgia Strait stock groups combined) stock groups were examined. When the results are summarized by two groups (US versus Canada) the bias of the estimates for each group are equal but of opposite sign and standard errors for each group are equal.

RESULTS

Simulation Set #1

The results of the twelve simulations which varied the ratio of Canadian to US stock groups while holding the relative contribution of stocks within each region of origin (Canada or US) constant (and approximately proportional to estimated run sizes) are summarized in Figure 1 and Appendix Tables 2a, 2b, and 2c. Estimates for the US stock group were similar by all three models. The largest difference among the models for the contribution by the US stock group at the same simulated stock mixture was 4%. Bias was similar among all three models for the US stock group. Except when the mixture samples were composed entirely of US stocks, biases for the US stock group were all ±7% or less. All three models overestimated the US stock group when its contribution was 20% or less to the mixture sample and underestimated the US stock group when its contribution was 60% or more to the mixture sample.

Biases for the Fraser River and Georgia Strait stock group estimates were generally smaller than for the US stock group. Over the range of Fraser River contributions examined (0% to 38%), biases for the Fraser River stock group were ±5% or less. Biases for the Georgia Strait stock group were similar to those for the Fraser River stock group. One exception was for the WDF model with 60%:36% composition of Georgia Strait:Fraser River stock groups which had the largest bias for the first set of simulations, -8%.

Simulation Set #2

The results of the four simulations which held the contributions of the US stock group constant at 4%, but varied the US stocks contributing to the 4% mixture, are summarized in Figure 2 and Appendix Tables 3a, 3b, and 3c. Estimates for the US stock group were similar by all three models. The largest difference among the models for the contribution by the US stock group at the same simulated stock mixture was 3%. Biases for the US stock group were all positive (the contribution of the US stock group was overestimated) and ranged from +3% to +7%. The WDF model had the largest bias (+7%) for the US stock group in the second set of simulations when the US stock group was composed of an equal mixture of the three US stocks and the smallest bias (+3%) when the US stock group was composed entirely of North Sound stocks. The two CDFO models had about the same degree of bias (+4%) for the estimates of the US stock group for all US stock mixtures examined in the second set of simulations.

Except when the US stock group was composed of a mixture of all three US stocks, biases for the Fraser River and Georgia Strait stock group estimates were negative or zero in all three models. The largest difference among the models for estimates of the Georgia Strait stock group at the same simulated stock mixture was 8% when all three US stocks were in the mixture sample.

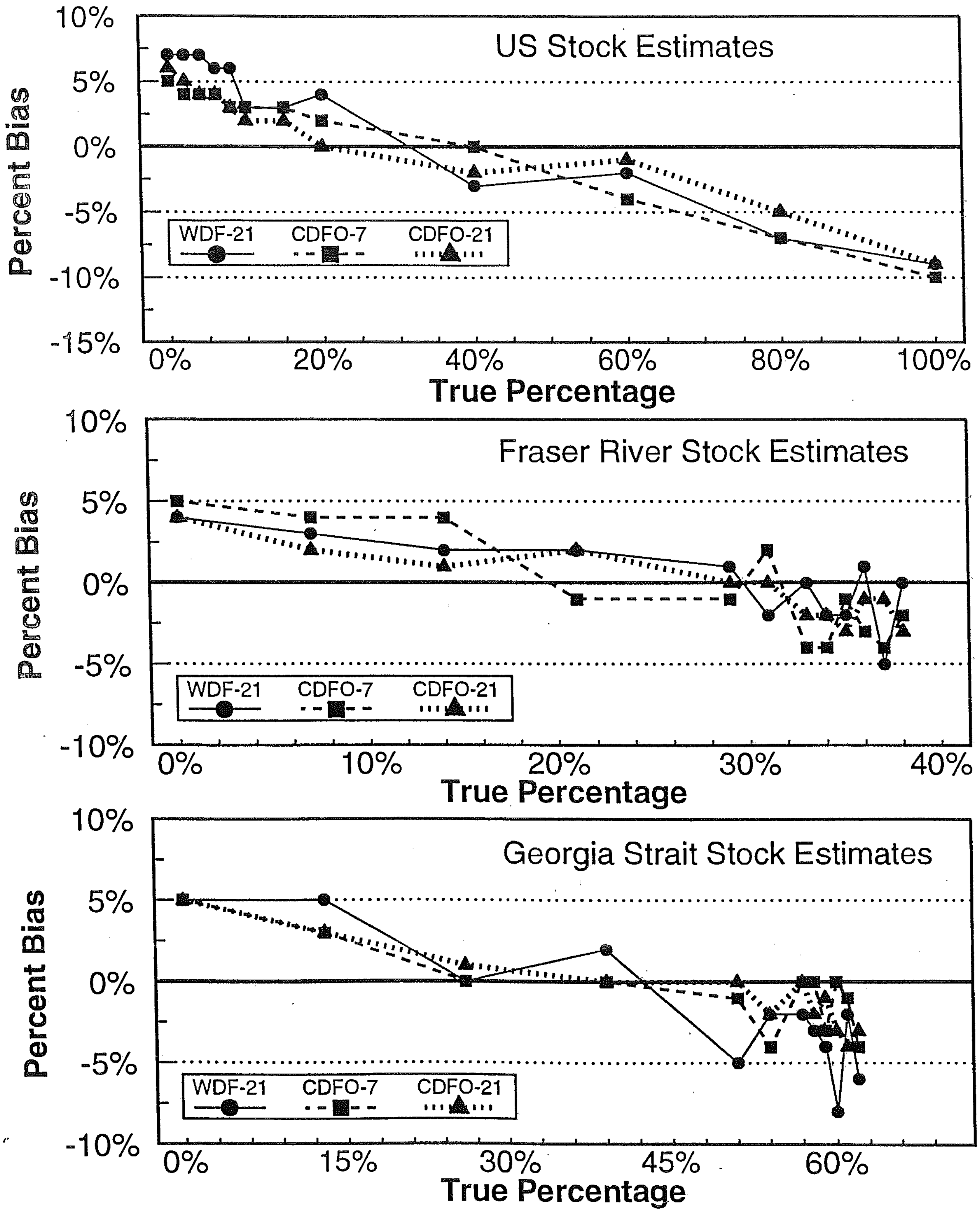

Figure 1. Percent bias (estimated percent - true percent) of estimates of US (Washington), Fraser River (FR), and Georgia Strait (GS) chum salmon stock groups from the first set of simulations (1 to 12) where the percentage contributions of the US stock group to the mixture samples were varied and the relative contribution of GS:FR stock groups held constant at 2:1.

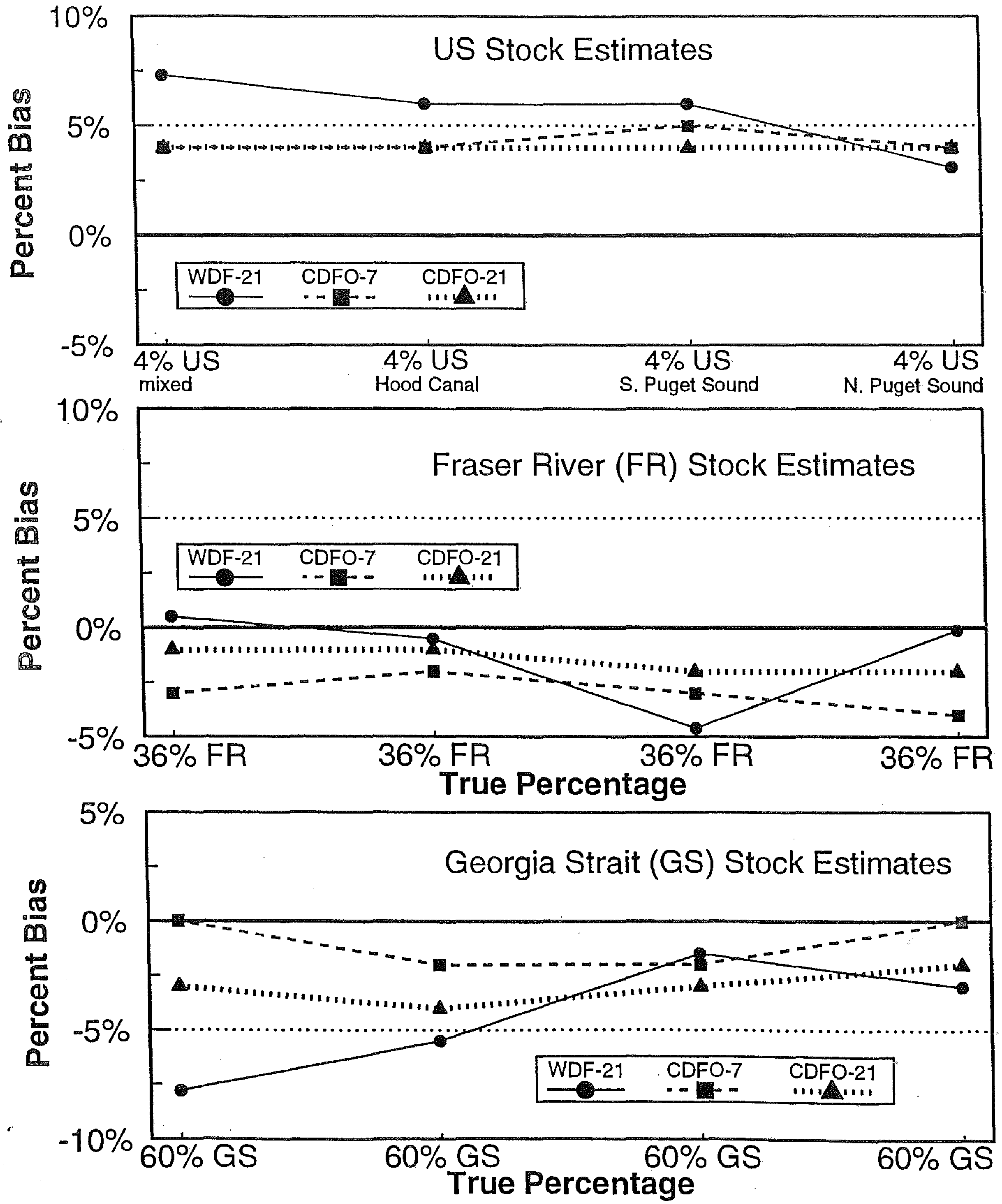

Figure 2. Percent bias (estimated percent - true percent) of estimates of US (Washington), Fraser River (FR), and Georgia Strait (GS) chum salmon stock groups from the second set of simulations (13 to 16) where the composition of the mixture sample was held constant at 4% US:36% FR:60% GS stock groups and the stocks contributing to the US stock group varied.

Simulation Set #3

The results of the six simulations which varied the contributions of the Fraser River:Georgia Strait stock groups while holding the total US and Canadian contribution constant are summarized in Figure 3 and Appendix Tables 4a, 4b, and 4c. The maximum difference among the models for estimates of the US stock group contribution at the same simulated stock mixture was 8%. This difference occurred between the WDF and CDFO 21-loci models with Fraser River:Georgia Strait stock group contributions of 77%:19%, respectively.

For the Fraser River stock group, biases in all three models were ±7% or less when the contribution of the Fraser River stock group was 48% or less. When the contribution of the Fraser River stock group exceeded 48% (77% and 96%), the largest biases of all 22 simulations were observed for the estimates of the Fraser River stock group (-7% to -18%). Biases for the Georgia Strait stock group were of similar size but opposite sign as the biases for the Fraser River stock group for simulations of the same mixture composition.

Effect of Size of the Mixture Sample

The results of simulations examining the effect of size of the mixture sample on the accuracy and precision of the stock composition estimates by the three models are shown in Figures 4 and 5 for the first stock mixture examined (0% US, 38% Fraser River, and 62% Georgia Strait). Although bias generally decreased as sample size increased there were some model and stock group specific exceptions. For the two CDFO models, bias decreased as sample size increased for the US and Georgia Strait stock groups. However, increased sample size did not decrease the bias of the CDFO 7-loci estimates of the Fraser River stock group (Figure 4). Increased sample size had the least effect on the bias of the estimates for the WDF model. Doubling the sample size from 200 to 400 decreased the bias of the estimates for the US and Georgia Strait stock groups by 1% for the WDF model and increased bias by 1% for the estimate of the Fraser River stock group. In comparison, going from a sample size of 200 to 400 reduced the bias of the CDFO 21-loci model by 2% or 3% for each of the three stock groups. Precision of the stock composition estimates for all three stock groups generally increased (the standard error decreased) as sample size increased (Figure 5).

The results of simulations examining the effect of size of the mixture sample are shown in Figures 6 and 7 for the second stock mixture examined (6% US, 35% Fraser River, and 59% Georgia Strait). The trend of a decrease in bias as sample size increased is evident but again there are some exceptions. For the CDFO 7-loci model, bias decreased as sample size increased for the US and Georgia Strait stock groups, however, as sample size increased bias of the estimates for the Fraser River stock group fluctuated (Figure 6). For the CDFO 21-loci model, all estimates for the contribution of the Georgia Strait stock group were within 2% of the actual stock composition and sample size had no obvious effect on bias. The WDF model had similar results for the Georgia

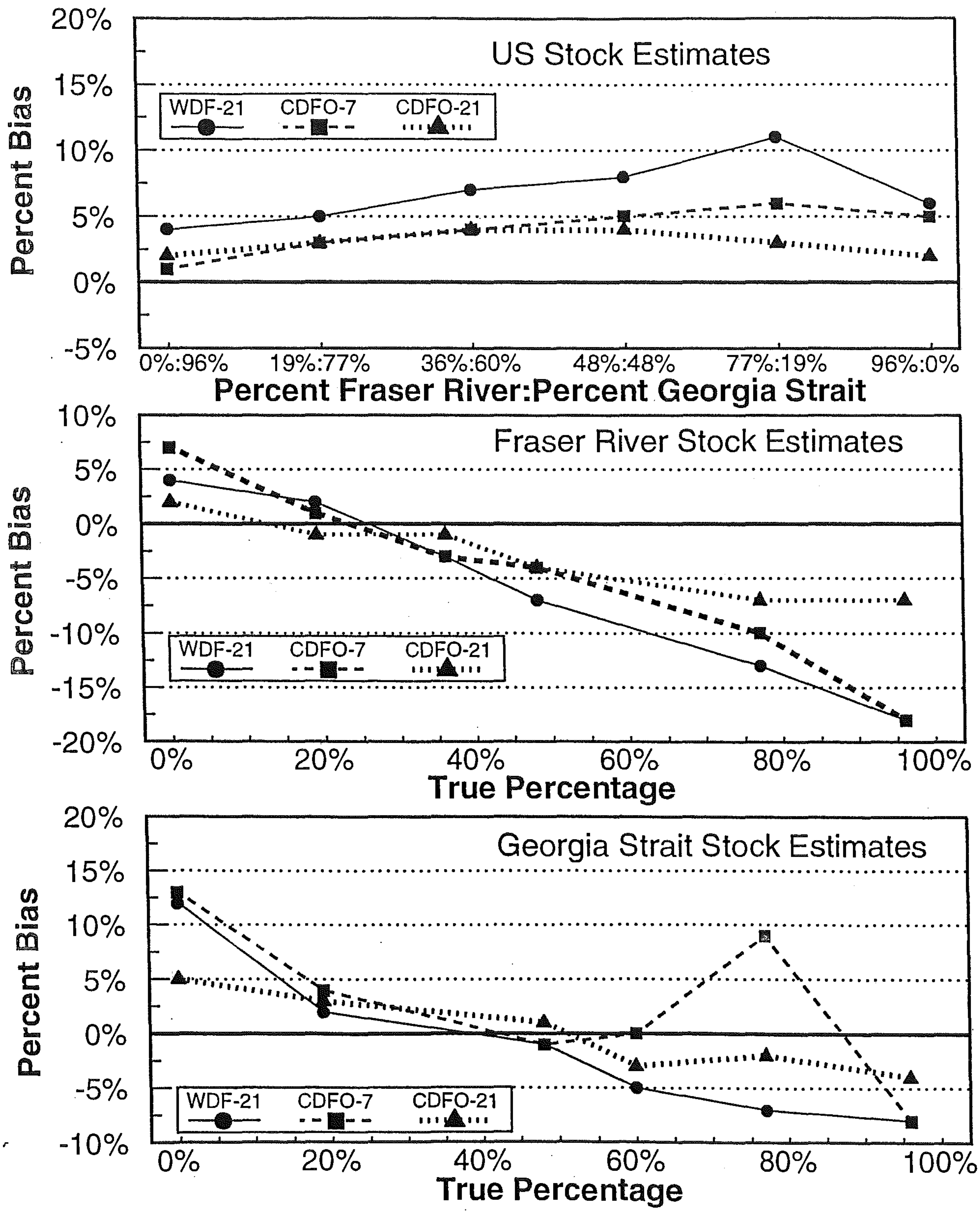

Figure 3. Percent bias (estimated percent - true percent) of estimates of US (Washington), Fraser River, and Georgia Strait chum salmon stock groups from the third set of simulations (17 to 22) where the percentage contributions of the Fraser River and Georgia Strait stock groups to the mixture samples were varied and the contribution of the US stock group held a constant 4%.

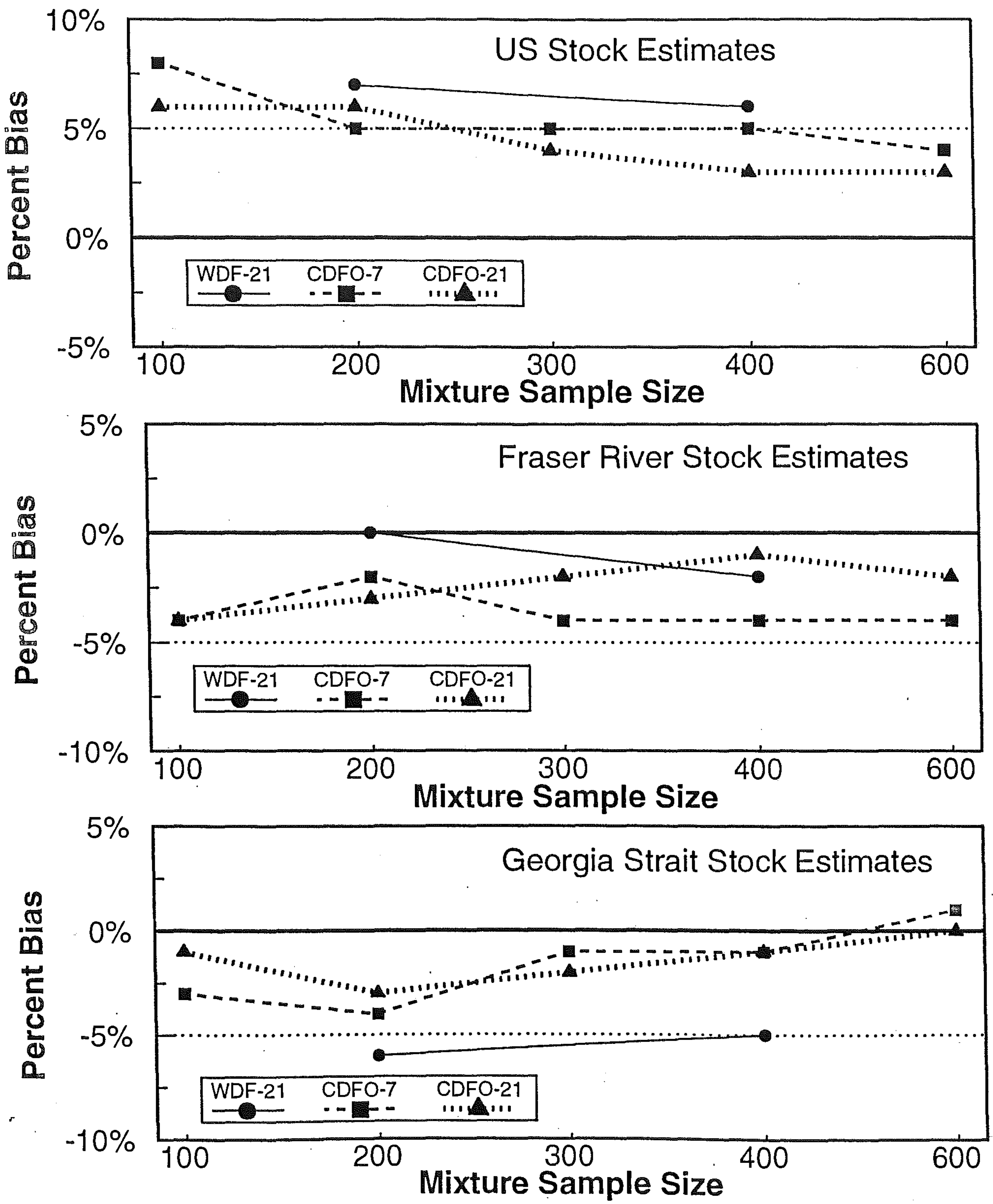

Figure 4. Percent bias (estimated percent - true percent) of estimates of US (Washington), Fraser River (FR), and Georgia Strait (GS) chum salmon stock groups for different mixture sample sizes and mixture samples composed of 0% US:38% FR:62% GS stock groups.

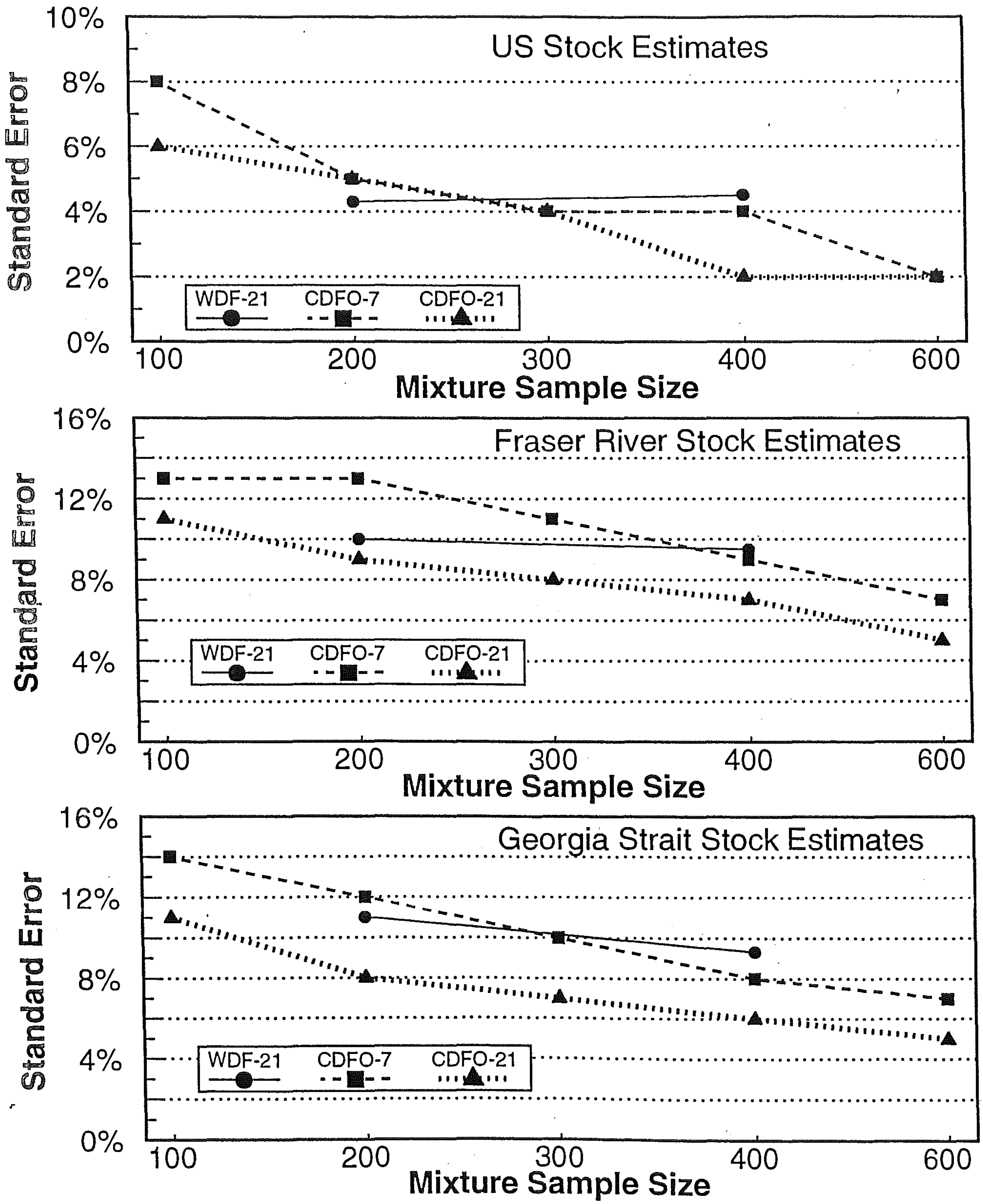

Figure 5. Standard error of estimates of US (Washington), Fraser River (FR), and Georgia Strait (GS) chum salmon stock groups for different mixture sample sizes and mixture samples composed of 0% US:38% FR:62% GS stock groups.

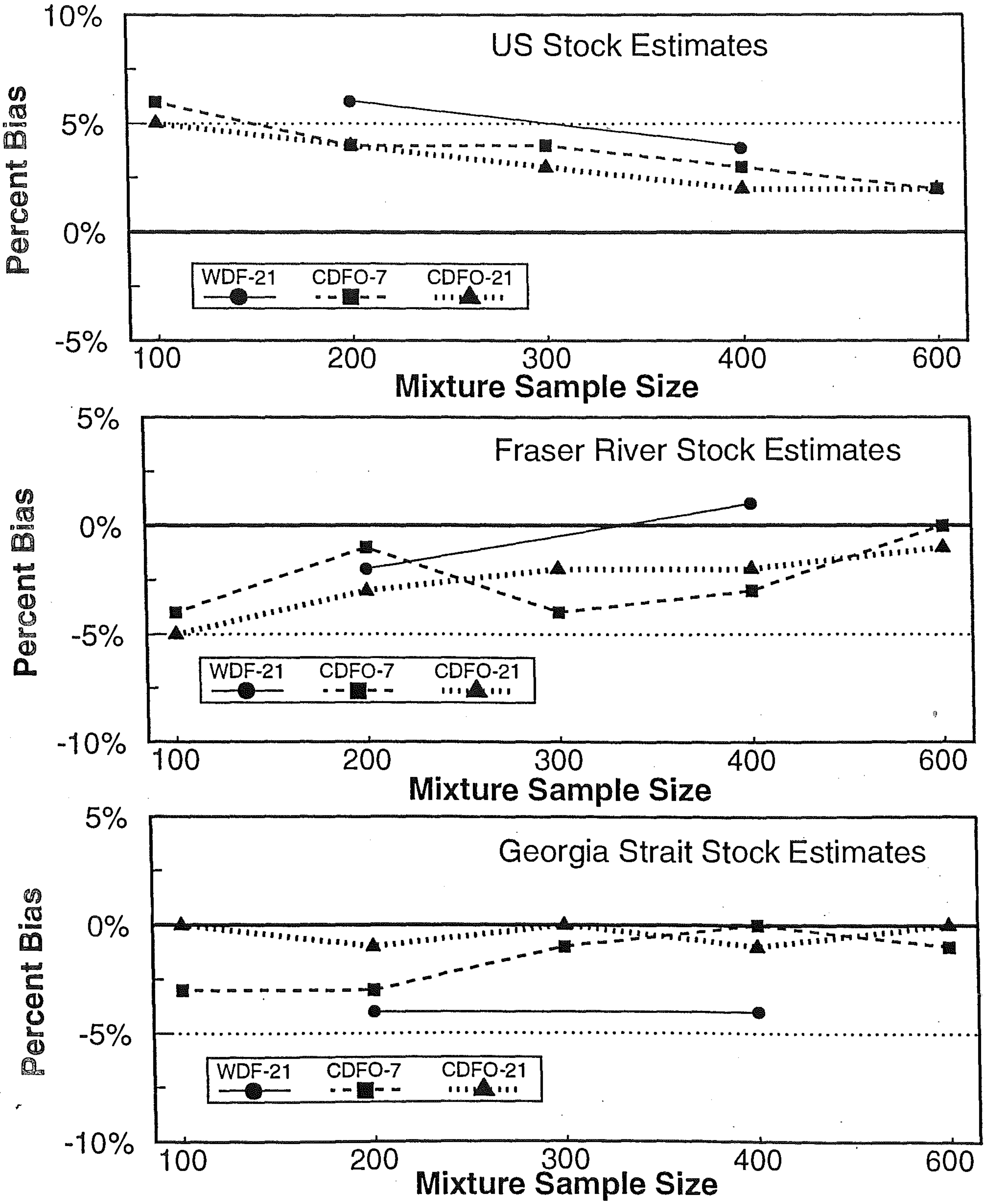

Figure 6. Percent bias (estimated percent - true percent) of estimates of US (Washington), Fraser River (FR), and Georgia Strait (GS) chum salmon stock groups for different mixture sample sizes and mixture samples composed of 6% US:35% FR:59% GS stock groups.

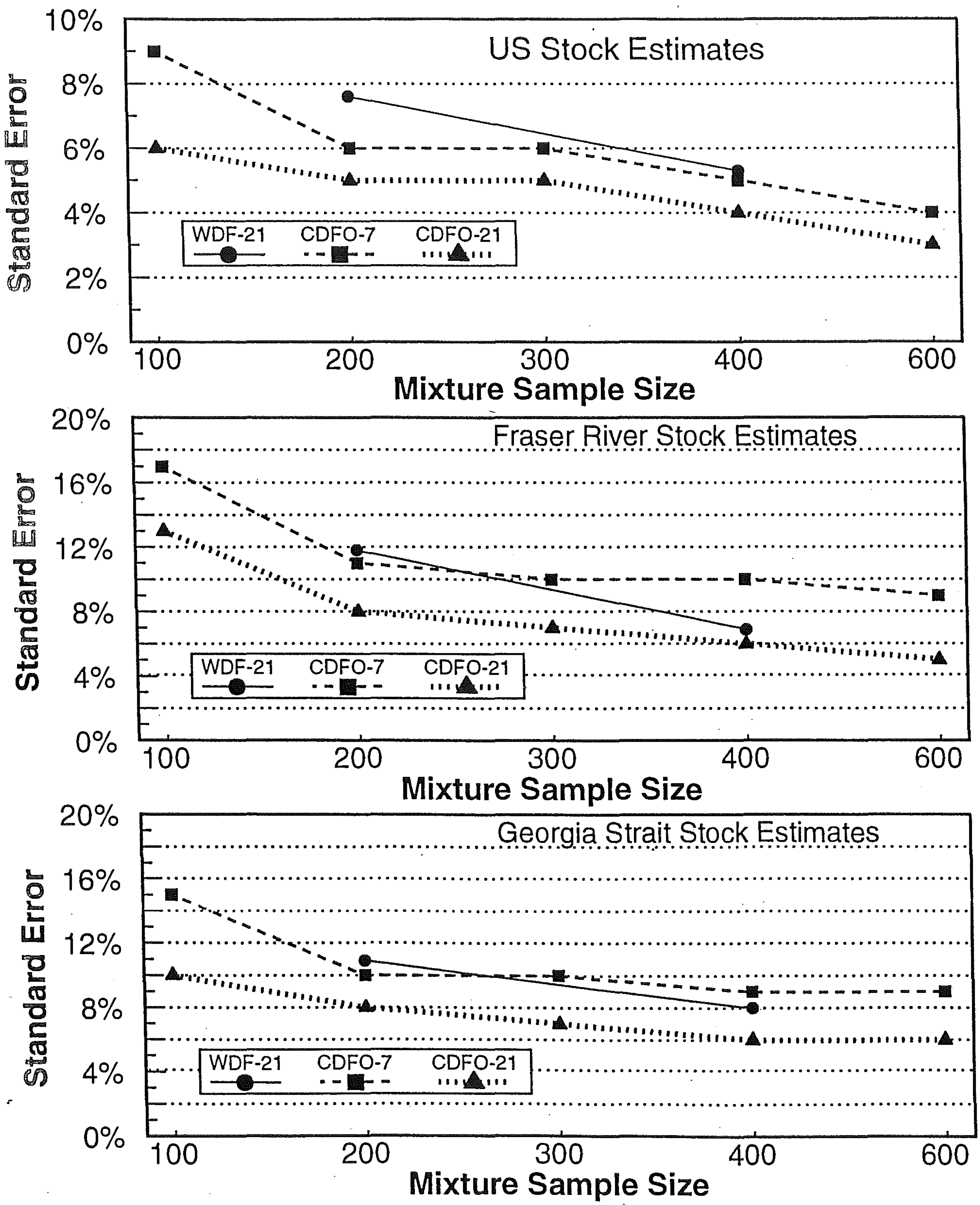

Figure 7. Standard error of estimates of US (Washington), Fraser River (FR), and Georgia Strait (GS) chum salmon stock groups for different mixture sample sizes and mixture samples composed of 6% US:35% FR:59% GS stock groups.

Strait stock group as an increase in sample size from 200 to 400 did not decrease the approximate -4% bias of the estimates. There was a general increase in the precision of the stock composition estimates (the standard error decreased) for all three stock groups in all three models as sample size increased (Figure 7).

Comparison of CDFO Models Using 7-loci and 21-loci Baselines

Biases for the estimates of the US stock group by the two CDFO models are compared in Figure 8 for the 22 simulations conducted with varied contributions of the US, Fraser River, and Georgia Strait stock groups to the mixture samples. Biases for the estimates of the total Canadian contribution (Fraser River and Georgia Strait combined) would be identical to those for the US stock group but of opposite sign. The largest difference between the two models for estimates of the contributions by the US or Canadian stock groups at the same simulated stock mixture was 3%.

Standard errors for the estimates of the US stock group by the two CDFO models are compared in Figure 9 for the 22 simulations conducted with varied contributions of the US, Fraser River, and Georgia Strait stock groups to the mixture samples. Standard errors for the estimates of the total Canadian contribution (Fraser River and Georgia Strait combined) would be identical to those for the US stock group. The largest difference between the two models for the standard errors of the estimates of the contributions by the US or Canadian stock groups at the same simulated stock mixture was 4%.

DISCUSSION

The major objective of this report was to quantify the accuracy and precision of the WDF 21-locus, 36-stock baseline (1989 version) used to estimate the stock composition of mixed-stock catches of chum salmon from northern Puget Sound (WDF Statistical Areas 7 and 7A). Three different models for estimating the stock composition of mixed-stock chum salmon catches using this baseline were examined. The models had differences in either their treatment of the baseline data, number of loci used in the analyses, and/or analytical methods. The simulations demonstrated that the baseline provides accurate estimates with acceptable precision for mixed-stock samples with stock compositions similar to those examined. Of the 198 stock group estimates from simulations 1 through 22 (22 simulations by 3 stock groups by 3 models), the absolute difference between the estimated stock group contribution and the true stock group contribution (i.e., the absolute bias) was 5% or less in 163 cases (82% of all estimates), between 6% and 10% in 29 cases (15% of all estimates), and greater than 10% in only 6 cases (3% of all estimates) .

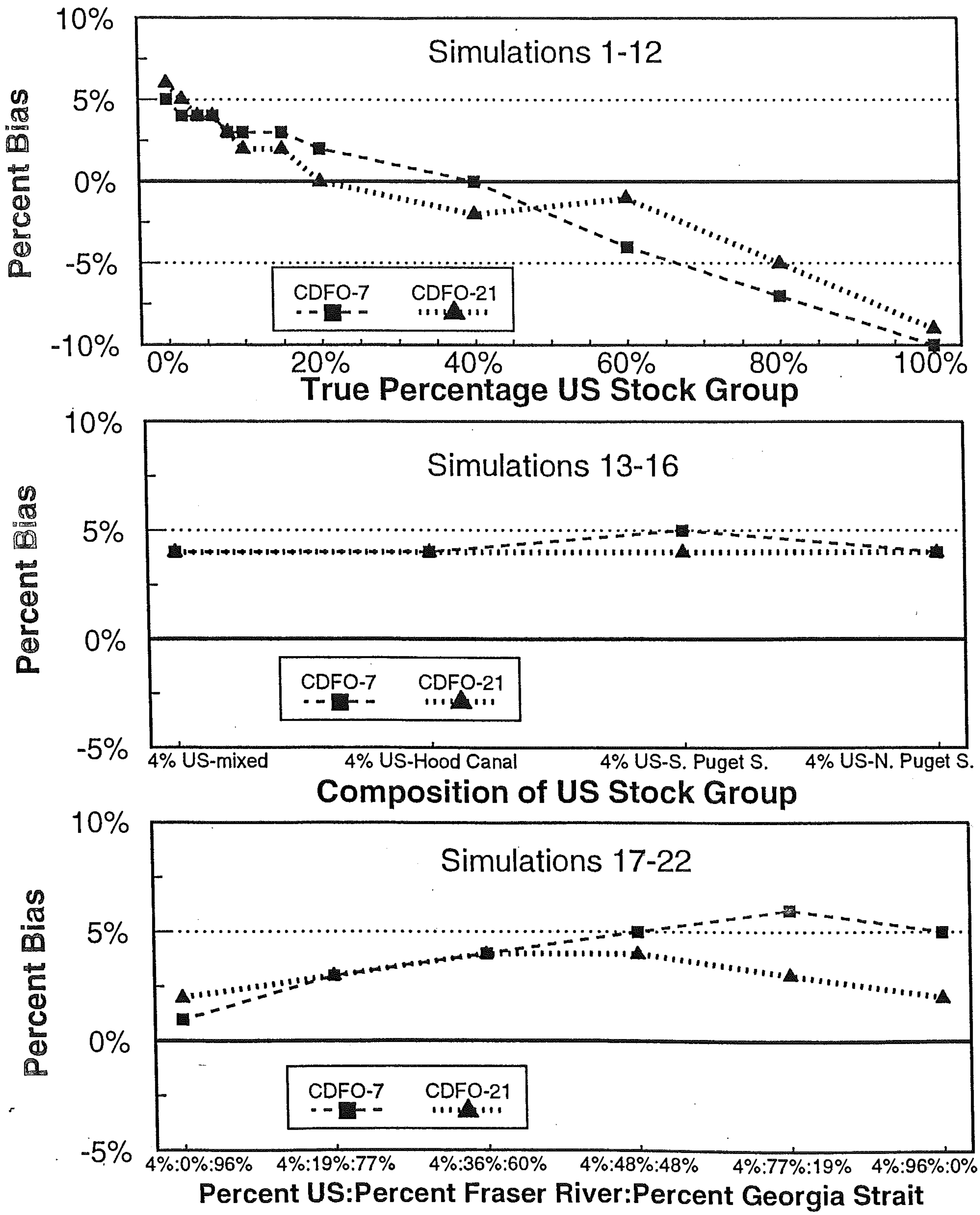

Figure 8. Comparison of percent bias (estimated percent - true percent) for estimates of the contribution by the US stock group for the CDFO models using either 7 or 21 loci for simulations 1 through 22 (described in text).

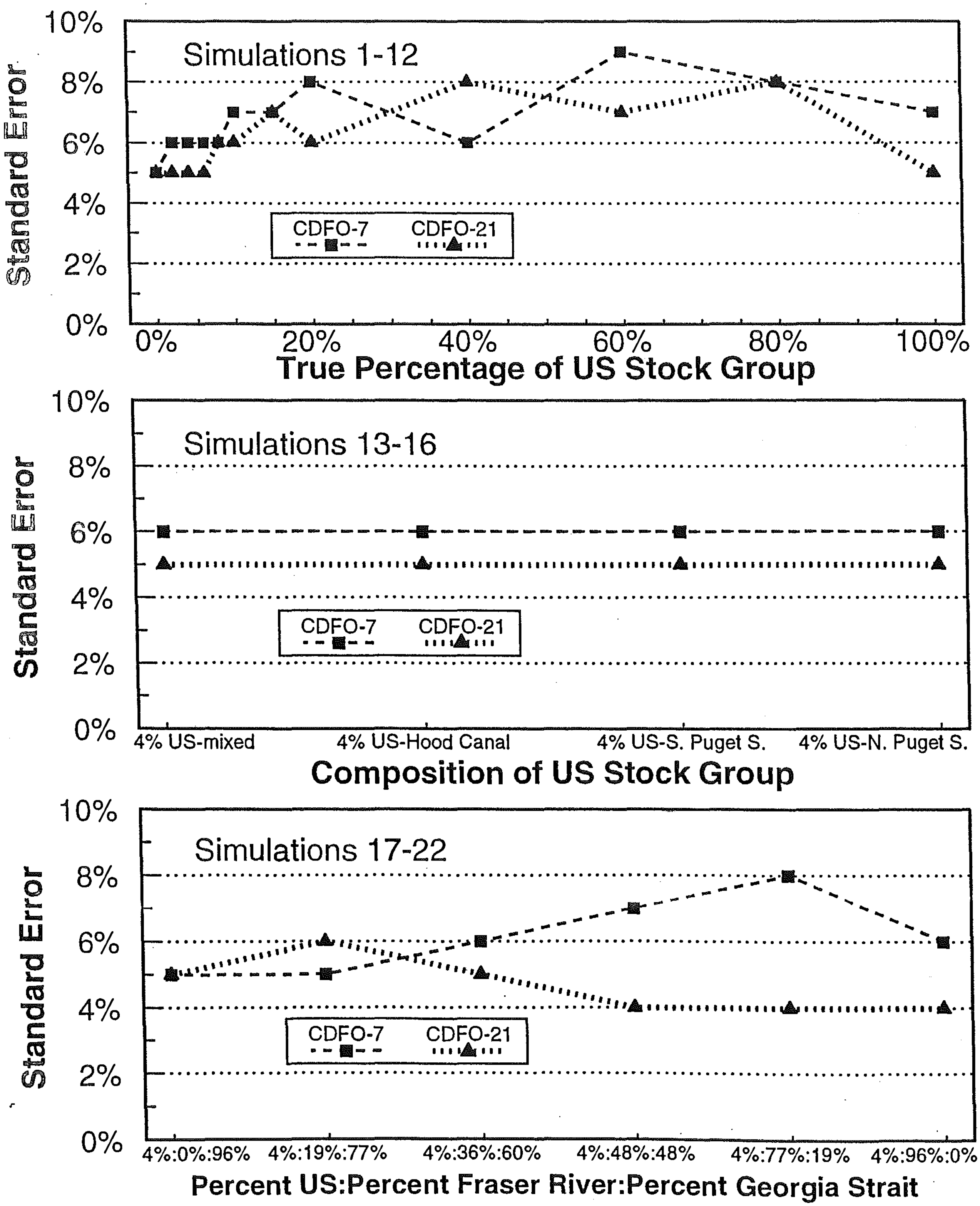

Figure 9. Comparison of standard error of the estimates of the contribution by the US stock group for the CDFO models using either 7 or 21 loci for simulations 1 through 22 (described in text).

Generally, the stock composition estimates produced using the WDF 21-locus, 36-stock baseline were shown to be insensitive to the model used. The three models examined had similar estimates of stock composition when analyzing similar stock mixtures. Of the 66 comparisons possible (22 simulations by 3 stock groups), the difference between the largest and smallest mean estimates for a stock group (US, Fraser River, or Georgia Strait) among the three models was 5% or less in 60 comparisons (91% of all comparisons). The difference between the largest and smallest estimates for a stock group among the three models was between 6% and 11% for the remaining 6 comparisons (9% of all comparisons).

All three models had similar points where the estimates for each stock group changed from consistently overestimating the contribution of a stock to consistently underestimating the contribution of a stock for the mixture samples analyzed. All three models overestimated the contribution of the US stock group when it composed 20% or less of the mixture sample and underestimated the contribution of the US stock group when it composed more than 20% of the mixture sample. Generally, all three models overestimated the contribution of the Fraser River stock group when it composed 30% or less of the mixture sample and underestimated the contribution of the Fraser River stock group when it composed more than 30% of the mixture sample. All three methods overestimated the contribution of the Georgia Strait stock group when it composed 50% or less of the mixture sample.

All three models produce similar estimates with comparable precision. For all three models, the largest biases of the estimates occurred when the contribution of one or two of the three stock groups was 20% or less to a mixture sample. All three models had relatively small bias when the three stock groups were each contributing 20% or more to a mixture sample (see the results of simulations 7, 8, 9, and 10).

It is important that the results of the simulations are not used to directly compare the WDF model to the two CDFO models to determine which model may be "best". The WDF model cannot be compared to the CDFO models because of differences among the models. Specifically, mixture samples were constructed using calculated multilocus genotypes (WDF) versus observed multilocus genotypes (CDFO) and estimates of stock composition, and associated standard errors, were made using 25 (WDF) versus 50 (CDFO) bootstrap replications. Because of the influence of these factors, the WDF model cannot be directly compared to the CDFO models. In one comparison (Craig Busack, WDF, Olympia WA, and Skip McKinnel, CDFO, Nanaimo BC, pers. comm.), it was demonstrated that given identical data for analysis, the maximum likelihood procedures used by the two agencies produce virtually identical estimates.

CONCLUSIONS

The simulations demonstrated that, for mixture samples with stock compositions similar to those used in the simulations for this report, the WDF 21-locus, 36-stock baseline (1989 version) provides accurate estimates of stock composition with acceptable precision when all three stock groups (US, Fraser River, and Georgia Strait) present in the mixture sample contribute 20% or more. *We conclude that the baseline provides adequate stock composition estimates for use by the Chum Technical Committee to fulfill Pacific Salmon Treaty obligations when contributions of the major stock groups are all greater than 20% for the fisheries simulated: northern Puget Sound (WDF Statistical Areas 7 and 7A) and southern Georgia Strait.* The stock composition estimates produced using this baseline were similar for the three models used for this report, especially when estimating total US versus total Canadian contributions.

However, when one or two of the three major stock groups contributed 20% or less to the mixture sample the estimates were biased and this bias was large relative to the estimates. This bias was present in all three models. *We recommend that methods of adjusting stock group estimates in this range (< 20%) to correct for bias be examined.*

Mixture sample sizes of 100 did not provide estimates with acceptable accuracy or precision. *The minimum sample size for a mixture sample should be 200 fish.*

SUMMARY

1. Simulations were conducted to quantify the accuracy and precision of the WDF 21-locus, 36-stock baseline (1989 version) used to estimate the stock composition of mixed-stock catches of chum salmon from northern Puget Sound. *The baseline provided accurate estimates with acceptable precision of the proportional contribution of US and Canadian chum salmon stock groups to mixed-stock samples with stock compositions representing commercial fisheries in northern Puget Sound and southern Georgia Strait.*

2. For the models and mixture compositions examined in this report, mixture sample sizes of 100 did not provide estimates with acceptable accuracy or precision. There was usually a large increase in precision (decrease in variance of the estimates) when the mixture sample size increased from 100 to 200. There were more modest gains in precision for increases in sample size over 200. *The minimum sample size for a mixture sample should be 200 fish.*

3. *For the simulations in this report, both 7-loci and 21-loci analyses provided accurate estimates of the total US and total Canadian contribution to mixed-stock samples representing commercial fisheries in northern Puget Sound and southern Georgia Strait.* In most cases, 21 loci provided more precise estimates of stock composition than 7 loci.

4. The largest bias of the stock contribution estimates occurred at extremes of stock composition, such as when one or more stock groups contributed 20% or less to the mixture sample. *We recommend that methods of bias correction be examined for mixture samples where one or more stock groups are estimated to contribute 20% or less to the sample.*

5. Three different GSI models were used in the simulations for this report. There were differences in the models' treatment of the baseline electrophoretic data, number of loci analyzed, analytical method used to estimate stock composition, and bootstrapping procedures used to estimate standard errors. *The three GSI models produced similar stock composition estimates when analyzing the same specified stock mixture, especially for the total US and total Canadian contribution.*

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