# 5.5 Development of production-scale genetic sexing techniques for routine catch sampling of Pacific halibut

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## Abstract

Pacific halibut is an important commercial species with an annual harvest valued at U.S. $100–170 million in the eastern portion of its range. Over the past four decades, size at harvest has declined dramatically (by ~20 pounds) and, coupled with sexually-dimorphic growth and size limits on commercial catches, suggests that commercial harvests are becoming increasingly female biased. Understanding the annual contribution of both sexes to the commercial harvest is important for predicting population dynamics and setting catch limits, but there is currently no reliable way to determine sex in the commercial harvest, given that halibut are eviscerated at sea. Here, we seek to develop high-throughput genetic assays for easy sex determination in Pacific halibut (*Hippoglossus stenolepis*), as well as perform comparative genomic analyses to closely related species. Restriction-site associated DNA sequencing resulted in the most loci ever identified in Pacific halibut: 40,308. Of these loci, 56 (with 70 single nucleotide polymorphisms) were linked to sex, and three loci were identified to persist exclusively in females. Taken together, evidence supported the previous hypothesis that sex determination in Pacific halibut is ZW determined. Comparisons of all loci identified in Pacific halibut with the closely related Atlantic halibut (*Hippoglossus hippoglossus*) and half-smooth tongue sole (*Cynoglossus semilaevis*) genome showed a high level of genomic similarity among these species. In addition, all loci linked to sex in the Pacific halibut were observed on a single chromosome, as is also true for both the Atlantic halibut and half-smooth tongue sole, which suggests that we have identified the sex-determining chromosome. High-throughput assays have been developed from a subset of sex-linked loci, and validation will be performed in the coming year.

## Introduction

Trends in mean weight-at-age, in concert with variance in underlying sex ratios and changing age-distribution over time, can have substantial effects on the demographics of fishery landings and influence population structure as individual cohorts progress through their fisheries. For eastern Pacific halibut (*Hippoglossus stenolepis*), the average individual weight of harvested fish is estimated to have varied more than two-fold over the last 80 years; increasing from approximately 20 pounds to over 40 pounds between the 1940s and the mid-1970s, then steadily declining to ~20 pounds by 2011 (Stewart and Monnahan 2016). In many regions, the largest decline was observed from 1995-2005 and was most strongly observed for age-10 halibut and older: age-classes primarily comprising the directed fishery (Stewart and Monnahan 2016). In conjunction with sexually-dimorphic growth, in which female halibut are typically larger at-age than males (Stewart and Monnahan 2016), longline selectivity tends to subject halibut to increased vulnerability to

harvest with increasing size (Stewart and Martell 2014). A minimum commercial size limit has remained constant since 1973 (Stewart and Monnahan 2016), resulting in an expectation that the sex composition of commercial catches has become increasingly female-biased over the last two to three decades. Given an assessment framework that predicts that both selectivity and natural mortality may vary according to sex (Stewart and Martell 2016), it is important to correctly estimate population sex ratios in order to conduct long-term policy analyses. For example, recent sensitivity analyses have indicated that uncertainty regarding sex ratios within commercial harvests can strongly influence our understanding of female spawning stock biomass ($SSB_f$), with 10% variance in estimated sex ratio translating into roughly 50 million pounds of estimated $SSB_f$ (I. Stewart, IPHC, unpublished). Such uncertainty may be exacerbated if age-specific sex compositions vary in space and time (*sensu* Clark 2004) as recent analyses suggest (Loher et al. 2016).

Unfortunately, there is presently no reliable way to determine the sex of commercially-harvested halibut at landing because they are eviscerated at sea. Efforts are underway to initiate a regular at-sea sex-marking program for the directed halibut fleet, in which retained catch would be marked by commercial fishers as either male or female during the dressing process (McCarthy 2015, Loher et al. 2017). Such a program would be conceptually similar to Atlantic lobster fisheries in which fishers "V-notch" gravid females prior to releasing them (Acheson and Gardner 2011) and add considerably to the IPHC's assessment and policy analyses. However, as such marks would not represent direct observations of sex, portside sampling would need to be accompanied by an empirical method to validate sex ratios as well as to monitor sex ratios within components of harvest for which at-sea marking might not be practical. Although sex in fishes may be determined by a variety of environmental factors (e.g., temperature; see review in Devlin and Nagahama 2002) and therefore not under strict genetic control, genetic sex-determination has been confirmed for the closely-related Atlantic halibut (*Hippoglossus hippoglossus*) (Tvedt et al. 2006) and nuclear microsatellite markers linked to sex have been found in Pacific halibut (Galindo et al. 2011). However, despite being extremely powerful for population studies due to their high variability, microsatellites are not ideal for production-scale sexing. Microsatellites have inherent problems with their reproducibility and high effort is required to screen variation in large samples; their routine application can be difficult and cost-prohibitive. A new set of molecular markers does not suffer from these problems: SNPs (single nucleotide polymorphisms), which are digital in nature (A, C, G or T) and are therefore highly reproducible. Furthermore, modern screening methods allow high throughput of SNPs at low costs. Indeed, SNPs are routinely used for real-time management of the Bristol Bay sockeye salmon (*Oncorhynchus nerka*) fishery (Dann et al. 2009), and recent advances in sequencing technologies have made the identification of SNPs in non-model species, such as Pacific halibut, feasible (Baird et al. 2008). The current report details progress on the development of a SNP-based sex assay intended to complement the IPHC's port sampling program. Development of the assay is expected to take two full years; the current document represents a one-year status report.

## Methods

### Sample collection

Samples were collected between 2003 and 2007 aboard IPHC-chartered longline vessels at five locations representing the IPHC-managed range of the species: from British Columbia (Haida Gwai) in the south to Attu Island in the western Aleutians and Pribilof Canyon in the southeastern Bering Sea; and at two additional sites (Adak Island and Petrel Bank) in the central Aleutians (Fig.1,

Table 1). Full details of sample collection can be found in Drinan et al. 2016. Briefly, for each halibut sampled, sex was determined via macroscopic gonad examination, the fish was measured to the nearest centimeter forklength, and its saggital otoliths and a tissue sample were collected. Tissue samples were preserved and stored in 100% ethanol.

**Laboratory techniques**

Single nucleotide polymorphisms were identified using restriction-site associated DNA sequencing (RADseq) techniques (Baird et al. 2008). RADseq is a reduced representation library technique that sequences individuals at thousands of loci spread throughout the genome, and is ideal for identifying genomic regions linked to phenotypic differences in species with few genomic resources. In this study, the *Sbf*-I restriction enzyme and standard laboratory techniques were used (Baird et al. 2008). Sequencing was performed on the Illumina HiSeq 4000.

**Development of baseline loci for Pacific halibut**

A baseline set of putative loci were identified using the *STACKS* v1.35 pipeline (Catchen et al. 2011, Catchen et al. 2013) and the sequence aligners *BOWTIE2* v2.1.0 (Langmead and Salzberg 2012) and *BLAST* v2.2.30 (Altschul et al. 1990). First, raw sequence reads were quality filtered and trimmed to 110 basepairs using *process_radtags*. Trimming was performed based on a decline in quality scores at the end of the reads. Next, loci present within individuals were identified (*ustacks*: -m 2, -M 3, -H, -r, -d, and --bound_high 0.03), and a catalog was created using the most deeply sequenced female and male from each stock (*cstacks*: -n 3). Loci identified within individuals were then compared to the catalog (*sstacks*) and genotypes were output (*populations*: -m 5, -r 0.25, and -p 3 [of 5]). A consensus sequence for each locus was identified to create a temporary database of putative loci.

Following the procedures outlined above, loci that were determined to align to repeat regions in the Pacific halibut genome, and loci with repeat elements within them, were filtered from the temporary database using the same alignment-based methodologies as those of Brieuc et al. (2014), using *BOWTIE2* (-end-to-end and --gbar 110) and *BLAST*. Loci that aligned exclusively to themselves using both aligners were retained as a final baseline of putative loci present in Pacific halibut.

**Genotyping of Pacific halibut**

To estimate genotypes of individuals at loci, PCR clones were first removed from the raw reads using *clone_filter* within *STACKS*. Next, reads were aligned to the putative set of loci using *BOWTIE2* and loci were identified within individuals (*pstacks*: -m 2 -p --bound_high 0.03). A catalog was created using the most deeply-sequenced female and male from each stock (*cstacks*: -g), and all individuals were compared to the catalog to identify loci present in each sample (*sstacks* and *populations*: -m 8 and -r 0.75). We further attempted to identify markers that exist only in one sex by re-running populations using different parameters (-m 4 and -r 0).

**Identification of sex linked markers**

Individuals were sorted into two groups based on sex. Genetic differentiation, measured by $F_{ST}$, was estimated between the groups using *Genepop* v4.2.1 (Raymond and Rousset 1995, Rousset 2008). Loci that were significantly differentiated and had an $F_{ST}$ value >= 0.30 were

deemed to be linked to sex. In addition, loci that were identified in at least 10 individuals in one sex and zero in the other sex were determined to be linked to sex.

### Comparative genomics and identification of sex determining chromosome

Sequence alignments between the baseline loci identified in this study and both the Atlantic halibut genetic map (Palaiokostas et al. 2013) and half-smooth tongue sole (*Cynoglossus semilaevis*) genome (Chen et al. 2014) were made to better understand the genomic similarities between species as well as identify the sex determining chromosome in Pacific halibut. Alignments were made using *BOWTIE2* using the same parameters as previously described.

### Development of high throughput assays

High-throughput TaqMan® assays (Thermo Fisher Scientific, Waltham, Massachusetts, USA) were developed from a subset of loci linked to sex. Forward and reverse reads for each sequence that aligned to the locus were merged using PEAR v0.9.6 (Zhang et al. 2014). A custom python script was created to calculate the proportional representation of each nucleotide at each base position for each locus. Nucleotides that existed in greater than 95% of sequences at a position were deemed the consensus. The consensus sequences were then used to create custom assays.

## Results

### Development of baseline loci for Pacific halibut

Sequencing resulted in 163,212,521 sequence reads, with an average of 1,542,009 reads per sample (standard deviation = 733763.1 reads). Development of a baseline set of putative loci resulted in the identification of 40,308 monomorphic and polymorphic loci. For comparison, all known previous genetic studies of Pacific halibut have utilized fewer than 100 loci (Nielson et al. 2009, Galindo et al. 2011, Drinan et al. 2016). The 40,308 loci identified in this study represent a major advance in genomic resources for Pacific halibut, and can be used as a baseline for future Pacific halibut genomics research.

### Genotyping of Pacific halibut and identification of sex linked loci

Individuals were genotyped at 38,276 loci (loci retained if > 50% of individuals genotyped), with an average of 35.8 (66%) females and 26.3 (64%) males genotyped per locus. The average $F_{ST}$ across loci was low (Fig. 2), with an average of 0.0015 (SD = 0.0262) for all loci. In total, 56 loci (70 SNPs) were identified as being linked to sex based on $F_{ST}$ (Fig. 2, Table 2). For all loci linked to sex, female genotypes were exclusively, or nearly so, heterozygous, while male genotypes were exclusively, or nearly so, homozygous. In addition, three loci were identified uniquely to females, while zero loci were identified uniquely to males. Taken together, these observations support the previous hypothesis that sex determination in Pacific halibut is determined in a ZW system (Galindo et al. 2011).

### Comparative genomics and identification of sex determining chromosome

There was a high degree of genome similarity between the Pacific halibut and both the Atlantic halibut and half-smooth tongue sole. In total, 4,442 loci aligned uniquely to the Atlantic halibut genetic map (78% of loci mapped in the Atlantic halibut genome). Loci aligned to all linkage groups (Fig. 3). Seven loci linked to sex in the Pacific halibut aligned uniquely to the Atlantic

halibut map. Six of these loci aligned to LG07 (linkage group 7), while one aligned to LG24 (Fig. 3; Table 2). In the Atlantic halibut, sex is determined in an XY system, and LG13 has been identified as the sex determining linkage group (Palaiokostas et al. 2013).

Alignment of the Pacific halibut baseline loci to the half-smooth tongue sole genome found 3,465 loci that aligned uniquely. Loci aligned to all chromosomes (Fig. 4; Table 2). Eight loci linked to sex in Pacific halibut aligned uniquely to the half-smooth tongue sole genome, and all aligned to the Z chromosome, which is a sex determining chromosome in the half-smooth tongue sole.

### Development of high throughput assays

Three loci were developed into high-throughput TaqMan® assays (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Two loci were selected based on SNP position (middle of the sequence), number of SNPs in the locus (fewer is preferred), and differentiation among males and females (greater differentiation is preferred). One additional locus was selected that was only observed in females and is hypothesized to persist on the W chromosome.

### Forthcoming in 2016-2017

Extensive validation of TaqMan® assays will be performed in 2016-2017. In addition, high-throughput, cost-effective DNA extraction techniques will be evaluated (Meeker*, et al.*, 2007). These will include an assessment of the quality of assays that are conducted using samples that are immediately preserved in ethanol upon collection relative to samples that are affixed to chromatography paper and stored dry (LaHood et al. 2008), the latter of which may be easier to invoke as a collection method within the IPHC's port sampling program.

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**Table 1. Samples used to identify sex-linked loci in Pacific halibut (*Hippoglosus stenolepis*).**

| Sample ID | Region | Sex | Year sampled | Number sequences |
|---|---|---|---|---|
| ANI_F001 | Adak Island | F | Winter 2007 | 1523218 |
| ANI_F004 | Adak Island | F | Winter 2007 | 815544 |
| ANI_F037 | Adak Island | F | Winter 2007 | 836724 |
| ANI_F040 | Adak Island | F | Winter 2007 | 798825 |
| ANI_F041 | Adak Island | F | Winter 2007 | 1489616 |
| ANI_F044 | Adak Island | F | Winter 2007 | 2448219 |
| ANI_F055 | Adak Island | F | Winter 2007 | 788500 |
| ANI_F072 | Adak Island | F | Winter 2007 | 1532014 |
| ANI_F084 | Adak Island | F | Winter 2007 | 2436878 |
| ANI_M015 | Adak Island | M | Winter 2007 | 485033 |
| ANI_M019 | Adak Island | M | Winter 2007 | 763154 |
| ANI_M026 | Adak Island | M | Winter 2007 | 1817549 |
| ANI_M028 | Adak Island | M | Winter 2007 | 680829 |
| ANI_M030 | Adak Island | M | Winter 2007 | 1297823 |
| ANI_M035 | Adak Island | M | Winter 2007 | 1783497 |
| ANI_M074 | Adak Island | M | Winter 2007 | 1770631 |
| ANI_M088 | Adak Island | M | Winter 2007 | 1973219 |
| ANI_M095 | Adak Island | M | Winter 2007 | 984159 |
| ATU_007_03 | Attu Island | F | Summer 2003 | 1683371 |
| ATU_008_03 | Attu Island | F | Summer 2003 | 1944845 |
| ATU_011_03 | Attu Island | F | Summer 2003 | 2317994 |
| ATU_015_03 | Attu Island | F | Summer 2003 | 1051345 |
| ATU_018_03 | Attu Island | F | Summer 2003 | 2282171 |
| ATU_024_03 | Attu Island | M | Summer 2003 | 1689686 |
| ATU_027_03 | Attu Island | F | Summer 2003 | 593185 |
| ATU_036_03 | Attu Island | M | Summer 2003 | 1115340 |
| ATU_038_03 | Attu Island | M | Summer 2003 | 641026 |
| ATU_047_03 | Attu Island | F | Summer 2003 | 1397809 |
| ATU_056_03 | Attu Island | M | Summer 2003 | 494830 |
| ATU_057_03 | Attu Island | M | Summer 2003 | 460269 |
| ATU_062_03 | Attu Island | F | Summer 2003 | 1035549 |
| ATU_066_03 | Attu Island | M | Summer 2003 | 2352957 |
| ATU_073_03 | Attu Island | F | Summer 2003 | 1616284 |
| ATU_082_03 | Attu Island | F | Summer 2003 | 696200 |
| ATU_084_03 | Attu Island | F | Summer 2003 | 1732880 |

**Table 1. (cont.)**

| | | | | |
|---|---|---|---|---|
| ATU_092_03 | Attu Island | M | Summer 2003 | 1329544 |
| ATU_095_03 | Attu Island | F | Summer 2003 | 441464 |
| PBC_w04_F004 | Pribilof Canyon | F | Winter 2004 | 628918 |
| PBC_w04_F010 | Pribilof Canyon | F | Winter 2004 | 1067269 |
| PBC_w04_F013 | Pribilof Canyon | F | Winter 2004 | 666272 |
| PBC_w04_F015 | Pribilof Canyon | F | Winter 2004 | 1421645 |
| PBC_w04_F020 | Pribilof Canyon | F | Winter 2004 | 1456832 |
| PBC_w04_F030 | Pribilof Canyon | F | Winter 2004 | 2342761 |
| PBC_w04_F032 | Pribilof Canyon | F | Winter 2004 | 1755481 |
| PBC_w04_F044 | Pribilof Canyon | F | Winter 2004 | 1920073 |
| PBC_w04_F045 | Pribilof Canyon | F | Winter 2004 | 2258631 |
| PBC_w04_F047 | Pribilof Canyon | F | Winter 2004 | 1420907 |
| PBC_w04_M006 | Pribilof Canyon | M | Winter 2004 | 764812 |
| PBC_w04_M008 | Pribilof Canyon | M | Winter 2004 | 1228857 |
| PBC_w04_M017 | Pribilof Canyon | M | Winter 2004 | 2384057 |
| PBC_w04_M023 | Pribilof Canyon | M | Winter 2004 | 1719299 |
| PBC_w04_M032 | Pribilof Canyon | M | Winter 2004 | 1027853 |
| PBC_w04_M038 | Pribilof Canyon | M | Winter 2004 | 1374064 |
| PBC_w04_M040 | Pribilof Canyon | M | Winter 2004 | 1176192 |
| PBC_w04_M046 | Pribilof Canyon | M | Winter 2004 | 1080581 |
| PBC_w04_M048 | Pribilof Canyon | M | Winter 2004 | 1899447 |
| PBC_w04_M054 | Pribilof Canyon | M | Winter 2004 | 1202353 |
| PTR_001_03 | Petrel Bank | F | Summer 2003 | 824419 |
| PTR_014_03 | Petrel Bank | F | Summer 2003 | 719855 |
| PTR_017_03 | Petrel Bank | M | Summer 2003 | 2167193 |
| PTR_020_03 | Petrel Bank | F | Summer 2003 | 714449 |
| PTR_023_03 | Petrel Bank | F | Summer 2003 | 1714475 |
| PTR_025_03 | Petrel Bank | F | Summer 2003 | 1424506 |
| PTR_028_03 | Petrel Bank | F | Summer 2003 | 1231783 |
| PTR_033_03 | Petrel Bank | M | Summer 2003 | 3408692 |
| PTR_035_03 | Petrel Bank | F | Summer 2003 | 2967598 |
| PTR_039_03 | Petrel Bank | F | Summer 2003 | 1487339 |
| PTR_042_03 | Petrel Bank | F | Summer 2003 | 1497975 |
| PTR_046_03 | Petrel Bank | M | Summer 2003 | 2642133 |
| PTR_050_03 | Petrel Bank | F | Summer 2003 | 1334289 |
| PTR_055_03 | Petrel Bank | F | Summer 2003 | 1121071 |
| PTR_059_03 | Petrel Bank | F | Summer 2003 | 1622675 |
| PTR_066_03 | Petrel Bank | F | Summer 2003 | 2731869 |
| PTR_084_03 | Petrel Bank | M | Summer 2003 | 1632595 |

**Table 1. (cont.)**

| | | | | |
|---|---|---|---|---|
| PTR_086_03 | Petrel Bank | F | Summer 2003 | 1417833 |
| PTR_092_03 | Petrel Bank | M | Summer 2003 | 797602 |
| QCI_F015 | Haida Gwaii | F | Winter 2004 | 1857832 |
| QCI_F033 | Haida Gwaii | F | Winter 2004 | 655661 |
| QCI_F045 | Haida Gwaii | F | Winter 2004 | 1550316 |
| QCI_F050 | Haida Gwaii | F | Winter 2004 | 1718563 |
| QCI_F052 | Haida Gwaii | F | Winter 2004 | 2626903 |
| QCI_F055 | Haida Gwaii | F | Winter 2004 | 1939012 |
| QCI_F060 | Haida Gwaii | F | Winter 2004 | 1158003 |
| QCI_F063 | Haida Gwaii | F | Winter 2004 | 1101979 |
| QCI_F064 | Haida Gwaii | F | Winter 2004 | 1575148 |
| QCI_F065 | Haida Gwaii | F | Winter 2004 | 1761755 |
| QCI_M016 | Haida Gwaii | M | Winter 2004 | 3826246 |
| QCI_M027 | Haida Gwaii | M | Winter 2004 | 2381103 |
| QCI_M047 | Haida Gwaii | M | Winter 2004 | 935023 |
| QCI_M057 | Haida Gwaii | M | Winter 2004 | 848040 |
| QCI_M059 | Haida Gwaii | M | Winter 2004 | 1840999 |
| QCI_M062 | Haida Gwaii | M | Winter 2004 | 2818033 |
| QCI_M064 | Haida Gwaii | M | Winter 2004 | 3825268 |
| QCI_M066 | Haida Gwaii | M | Winter 2004 | 2783909 |
| QCI_M075 | Haida Gwaii | M | Winter 2004 | 1930246 |

**Table 2. Loci linked to sex in Pacific halibut (*Hippoglossus stenolepis*) and alignment locations in Atlantic halibut (*Hippoglossus hippoglossus*) linkage map (Palaiokostas et al. 2013) and half-smooth tongue sole (*Cynoglossus semilaevis*; Chen et al. 2014).**

| Locus name | *H. stenolepis* SNP position | *H. stenolepis* $F_{ST}$ | *H. hippoglossus* chromosome | *H. hippoglossus* position (cM) | *C. semilaevis* chromosome | *C. semilaevis* position (bp) |
|---|---|---|---|---|---|---|
| Hs10183 | 92 | 0.4679 | | | | |
| Hs10684 | 71 | 0.3872 | | | | |
| Hs11134 | 54 | 0.4349 | | | | |
| Hs11686 | 67 | 0.4513 | | | | |
| Hs1230 | 16 | 0.4861 | | | | |
| Hs13544 | 55 | 0.4576 | | | | |
| Hs15721 | 106 | 0.4494 | | | | |
| Hs16176 | 55 | 0.3885 | | | | |
| Hs16264 | 86 | 0.4484 | LG07 | 24.8 | | |
| Hs16541 | 31 | 0.4459 | LG07 | 24.8 | | |
| Hs16541 | 50 | 0.4459 | LG07 | 24.8 | | |
| Hs16978 | 56 | 0.4516 | | | | |
| Hs17396 | 102 | 0.4182 | | | Z | 9643980 |
| Hs17538 | 77 | 0.4413 | | | | |
| Hs19071 | 72 | 0.4532 | | | Z | 7842628 |
| Hs19133 | 58 | 0.436 | | | | |
| Hs1940 | 93 | 0.3449 | | | | |
| Hs19521 | 60 | 0.3353 | | | | |
| Hs19887 | 72 | 0.4008 | | | | |
| Hs22289 | 99 | 0.3791 | | | | |
| Hs22356 | 16 | 0.3587 | LG07 | 28.1 | | |
| Hs22356 | 17 | 0.3587 | LG07 | 28.1 | | |
| Hs23885 | 27 | 0.4667 | LG07 | 0.8 | | |
| Hs26040 | 44 | 0.4507 | | | | |
| Hs26580 | 81 | 0.4393 | | | Z | 17732131 |
| Hs26580 | 104 | 0.4353 | | | Z | 17732131 |
| Hs26580 | 105 | 0.4449 | | | Z | 17732131 |
| Hs26580 | 107 | 0.4408 | | | Z | 17732131 |
| Hs26674 | 33 | 0.4727 | | | Z | 5950860 |
| Hs2713 | 23 | 0.4667 | | | Z | 16880499 |
| Hs2806 | 54 | 0.3387 | | | | |
| Hs29837 | 11 | 0.457 | | | | |
| Hs31532 | 39 | 0.4532 | | | | |

**Table 2. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Hs32225 | 42 | 0.4467 | | | | |
| Hs32939 | 31 | 0.4583 | | | | |
| Hs32939 | 74 | 0.4583 | | | | |
| Hs33058 | 8 | 0.4515 | LG24 | 61.8 | Z | 7979068 |
| Hs33058 | 64 | 0.4515 | LG24 | 61.8 | Z | 7979068 |
| Hs35909 | 65 | 0.3047 | | | | |
| Hs3615 | 103 | 0.4724 | | | | |
| Hs37509 | 46 | 0.4473 | | | | |
| Hs37971 | 92 | 0.4143 | | | | |
| Hs37971 | 102 | 0.4143 | | | | |
| Hs37971 | 104 | 0.4101 | | | | |
| Hs39920 | 36 | 0.3074 | | | | |
| Hs40015 | 83 | 0.4867 | | | | |
| Hs41758 | 93 | 0.4258 | | | | |
| Hs42000 | 68 | 0.3745 | | | | |
| Hs4502 | 8 | 0.4331 | | | | |
| Hs4531 | 48 | 0.4376 | | | | |
| Hs45763 | 78 | 0.4344 | | | | |
| Hs45763 | 86 | 0.4344 | | | | |
| Hs487 | 104 | 0.4643 | | | | |
| Hs50887 | 104 | 0.4444 | LG07 | 29.7 | | |
| Hs53533 | 89 | 0.4605 | | | | |
| Hs54452 | 40 | 0.4025 | | | Z | 11663080 |
| Hs5585 | 48 | 0.342 | | | | |
| Hs6189 | 44 | 0.4771 | | | | |
| Hs6347 | 72 | 0.4879 | | | | |
| Hs7772 | 39 | 0.4041 | | | | |
| Hs7949 | 30 | 0.3715 | LG07 | 13 | | |
| Hs7949 | 47 | 0.3715 | LG07 | 13 | | |
| Hs7949 | 57 | 0.3715 | LG07 | 13 | | |
| Hs7949 | 66 | 0.3715 | LG07 | 13 | | |
| Hs7949 | 82 | 0.4839 | LG07 | 13 | | |
| Hs8831 | 104 | 0.3868 | | | | |
| Hs8882 | 82 | 0.3977 | | | Z | 7344975 |
| Hs9030 | 48 | 0.492 | | | | |
| Hs9050 | 9 | 0.4402 | | | | |
| Hs9788 | 73 | 0.4648 | | | | |

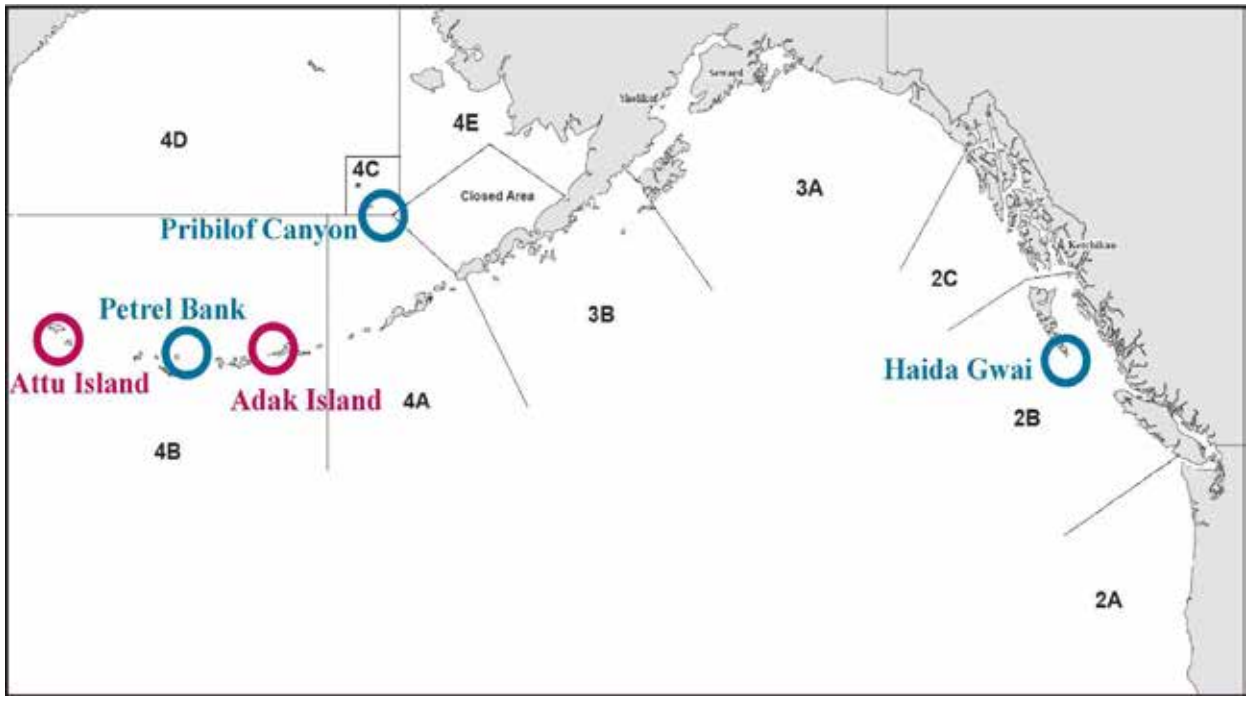


**Figure 1. Locations at which the Pacific halibut contained in this study were sampled. Locations depicted in red were sampled during the summer (i.e., on halibut feeding grounds) and those in blue during the winter (on spawning grounds).**

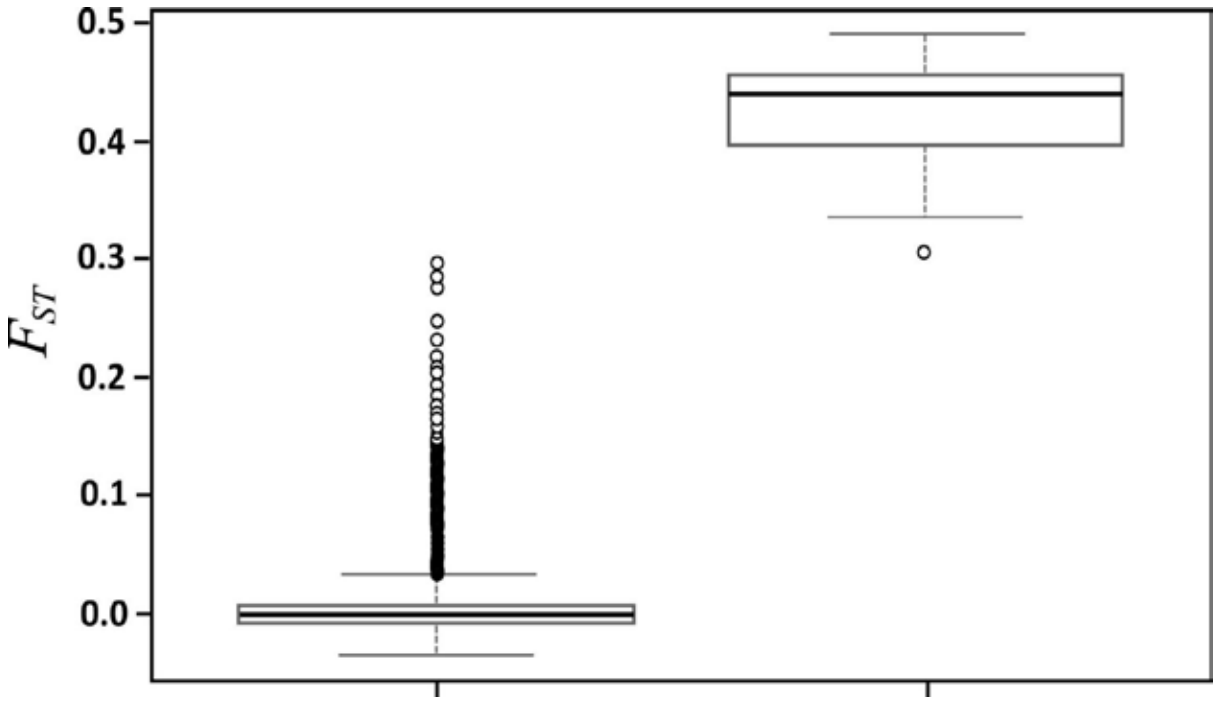


**Figure 2. Boxplots of genetic differentiation ($F_{ST}$) between the sexes in Pacific halibut (*Hippoglossus stenolepis*) at markers linked to sex and those not linked to sex.**

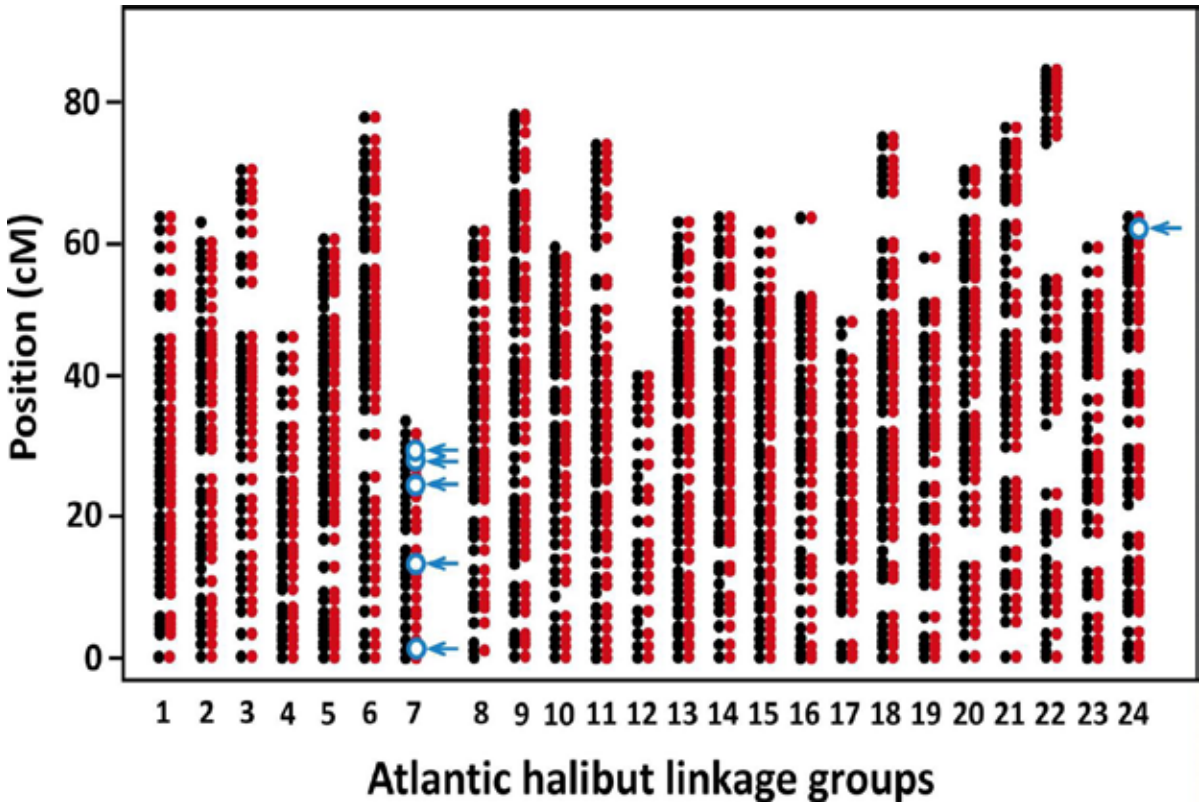


**Figure 3. Comparative genomics between Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*Hippoglossus stenolepis*). The x-axis represents linkage group (proxy for chromosome) and the y-axis is position in the linkage group (in centimorgans). Black dots represent loci mapped in Atlantic halibut and the red dots represent loci found in Pacific halibut. Dots next to each other represent the same locus observed in both species. * represent loci linked to sex in the Pacific halibut.**

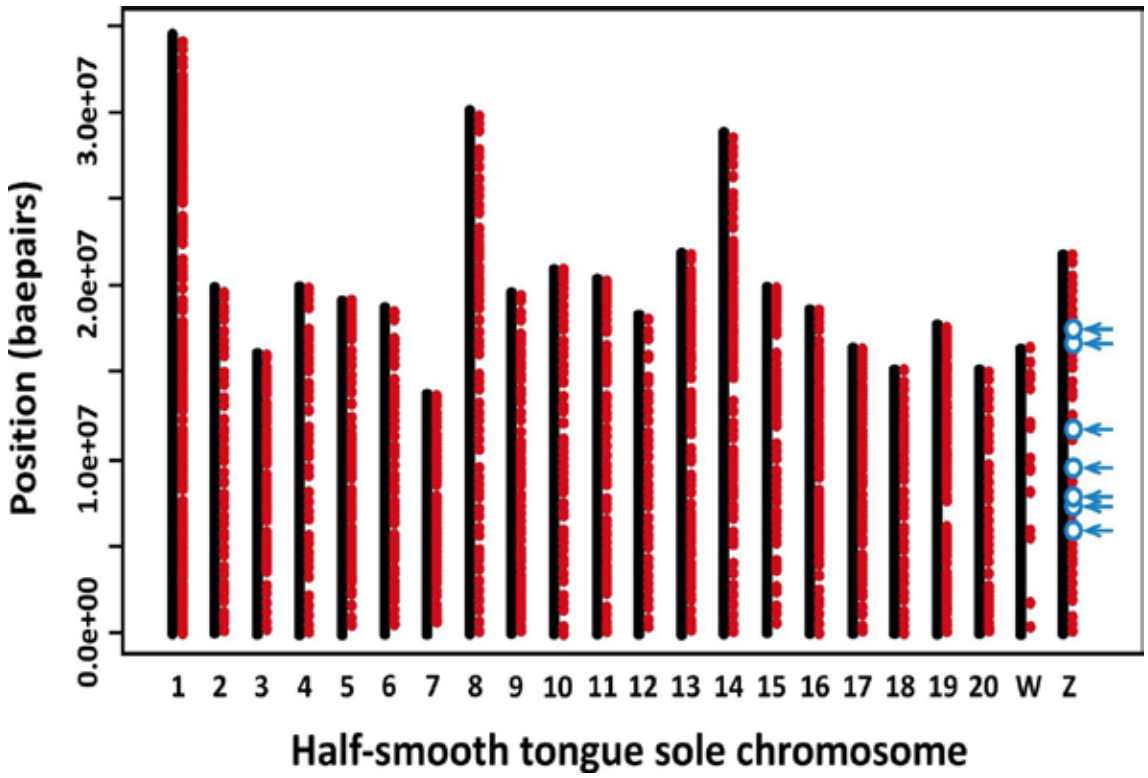


**Figure 4. Comparative genomics between half-smooth tongue sole (*Cynoglossus semilaevis*) and Pacific halibut (*Hippoglossus stenolepis*). The x-axis represents linkage group (proxy for chromosome) and the y-axis is position in the linkage group (in centimorgans). Black dots represent loci mapped in half-smooth tongue sole and the red dots represent loci found in Pacific halibut. Dots next to each other represent the same locus observed in both species. * represent loci linked to sex in the Pacific halibut.**