2.9 Voluntary at-sea sex marking of Pacific halibut in the targeted longline fleet

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Abstract

Uncertainty regarding the sex ratio of commercial Pacific halibut catches represents one of the largest sensitivities within the current Pacific halibut stock assessment, in particular generating considerable variability around estimates of total female spawning biomass. Pacific halibut demonstrate a variety of behavioral and seasonal characteristics that likely allow fishers to selectively target one sex over the other, resulting in the potential for large amounts of catch to come from times and places in which the population's underlying sex ratio is highly skewed. In order to monitor sex ratios within the commercial catch and more accurately model population characteristics, the International Pacific Halibut Commission (IPHC) has embarked upon a program designed to generate and validate observational data from the landed catch within its existing port sampling program. This program would be composed of a combination of at-sea marking in which commercial fishers mark each halibut as either male or female as the fish are dressed and, at offload, IPHC port samplers then record the marked sexes and collect tissue samples for potential future genetic analysis. The program was officially launched in 2014 with the development of methods to mark Pacific halibut at sea; a small pilot test of those marking methods was conducted in 2015 with the cooperation of vessels operating out of the port of Homer, Alaska; and in 2016, voluntary at-sea marking was conducted by the commercial fleet throughout British Columbia. Fleet involvement during 2016 was excellent, and feedback from fishers and processors was positive, with the two groups respectively reporting that marking was not disruptive of normal fishing activity, nor did it have any adverse effects on marketability of the fish. Voluntary at-sea marking will be increased in scale in 2017 and 2018 so as to represent all IPHC-sampled ports in all regulatory areas, with the ultimate goal of having a routine data collection procedure in place for the 2019 fishing season, composed of a combination of at-sea sex marking by the commercial fleet in conjunction with limited genetic validation.

Introduction

After peaking at approximately 40 pounds (18 kg) in the mid-1970s, the average size of Pacific halibut (*Hippoglossus stenolepis*; hereafter "halibut") captured in the commercial longline fishery declined for more than three decades, to just over 20 pounds (9 kg) by 2010 (Stewart and Monnahan 2016). This trend was first documented in the scientific literature more than 15 years ago (Clark et al. 1999, Clark and Hare 2002). Shortly thereafter, it generated concerns regarding the International Pacific Halibut Commission (IPHC) staff's understanding of sex ratios and sex-specific mortality within the commercial fishery (Clark 2004) given that female Pacific halibut grow faster, and therefore recruit to the commercially fishery at younger age, than males; and that the sex ratio of the commercial catch cannot be determined using direct observations because commercially-harvested Pacific halibut are dressed (eviscerated) at sea. Initially, the greatest concern was that sex ratios within the commercial fishery were likely to become increasingly female over time. Whereas in the mid-1980s commercial catches were estimated to be composed of sex ratios relatively similar to those found in the underlying population (Clark 2004), by 2006 the average age at which females were being harvested was estimated to be three years younger than that of males (Hare and Clark 2006) and it had even been suggested that a considerable proportion of male stock might never reach sizes associated with full fishery selectivity (Clark and Hare 2006; see also the age distributions of halibut of commercially-sublegal size (i.e., <32" (82cm) forklength) in Stewart 2017). Such dynamics are particularly relevant to the IPHC's harvest policy, which is structured to adjust target harvest rates based on female spawning biomass in relation to a threshold and limit reference point (Stewart 2016). To assist in modeling female biomass, Clark (2004) developed a method for estimating the sex ratio of the commercial catch based on observed size at age. In effect, large, young halibut possess a high probability of being female and small, old halibut a high probability of being male. By extension, at any combination of length and age the probability of a halibut's sex may be calculated and these probabilities can then be applied to the unsexed commercial age samples in order to estimate their sex composition (Clark 2004). However, even at the outset there were reasons to be concerned about the accuracy of this method, and even in the absence of long-term or directional changes in size at age, regional and seasonal changes in the sex ratio experienced by the fleet can complicate our ability to estimate how fishing mortality might impact the stock.

Fundamentally, Clark's (2004) estimator is based on a different quantity than we are ultimately interested in: the survey catch, not the fishery catch itself. The dynamics of the fishery can differ from those of the IPHC fishery-independent setline survey (setline survey) in important ways. The setline survey spans only ~40% of the commercial fishing period, whereas seasonal migration has the potential to strongly influence the redistribution of halibut biomass (Leaman et al. 2002, Loher and Blood 2009), including while the fishery is open in late spring and early autumn (Loher and Clark 2010, Loher 2011). The fishery may also target specific stock components or geographic areas, generating potential for an unknown degree of bias in commercial landings composition relative to setline survey catches. This could occur if fishers are able to selectively target one sex over the other (in this case, targeting the larger females), by taking advantage of differences in either sex-specific selectivity or spatial distribution. As such, seasonal and behavioral patterns of both the halibut and the fleet during the course of annual fishing seasons might lead to large amounts of the catch coming from times and places in which the sex ratio is highly skewed. The degree to which this might be true remains relatively unknown because we have not been able to directly measure such dynamics. However, we do know that the average size of fish comprising commercial landings may be larger than that of setline survey catches (Hare 2010) and that Pacific halibut aggregate according to sex and size on small spatial scales (Loher and Hobden 2012). Both sex-specific and seasonally-variable selectivity has been demonstrated in other commercially-exploited species, including Tasmanian calamari (*Sepioteuthis australis*; Hibberd and Pecl 2007) and groupers (Serranidae) in the Gulf of Mexico (Coleman et al. 1996, Aguilar-Perera 2006). Similarly, a comparison of the known sex composition of Pacific halibut catches observed during commercial longline trips in 2010 and 2011 with the expected sex ratios of those offloads based on setline survey data demonstrated considerable differences between the two methods, and evidence that such differences were greatest outside of the summer setline survey period (Loher et al. 2016).

In the absence of observational sex-ratio data for the commercial fishery, the 2013 stock assessment was found to be quite sensitive to the assumption that relative selectivity-at-age for the oldest males and females was equivalent in the setline survey and commercial fishery (Stewart and Martell 2014). A 20% range in fishery selectivity sex-ratio was found to translate into a range of female spawning biomass estimates that equaled about 25% of the estimated total spawning biomass; that is, roughly 50 million pounds (22,680 metric tons) of variance. Renewed exploration of the sensitivity of the assessment to sex ratio using more recent assessment results suggests a similar sensitivity despite the inclusion of several additional years of data and some model improvements (Stewart and Hicks 2017). Without direct observations of the sex of commercially-harvested halibut there is no way to determine the magnitude of this uncertainty and/or bias in spawning stock estimates that is included in current assessment results. As such, the IPHC initiated a sampling program in 2014 to introduce at-sea sex-marking into the directed Pacific halibut fishery, in which retained catch would be routinely marked by commercial fishers as either male or female during the dressing process and those marks would be recorded by IPHC port samplers as they collect length data and otoliths for aging. Such a program is conceptually similar to that which is used in Atlantic lobster (*Homarus americanus*) fisheries in which fishers "V-notch" the tails of all egg-bearing females before releasing them (Acheson and Gardner 2011). Routine marking of Pacific halibut by the commercial fleet would add considerably to the IPHC's assessment and harvest policy development as well as providing a direct avenue for fishers to contribute to the scientific understanding of the halibut stock. This document outlines the program's progress to date and its planned course for the future.

Progress to date

The IPHC's at-sea sex marking and validation program was formally launched in 2014 and is designed to be accomplished in seven stages over a five-year period, culminating in the incorporation of sex-mark data collection into routine port sampling for commercial size and age data beginning during the 2019 commercial Pacific halibut fishing season:

1) Development of methods to mark Pacific halibut at sea, conducted on the IPHC's setline survey platform (2014).
2) Pilot-testing of the chosen marking methods in a limited commercial setting (2015).
3) Initiation of voluntary at-sea marking by the commercial fleet within a single IPHC regulatory area (2016).
4) Development of a genetic sexing assay for validating sex ratios in port samples and determining sex in unmarked harvest sectors (2016-17).
5) Scale up of voluntary at-sea marking by the commercial fleet to include all IPHC regulatory areas (2017-18).
6) Examination of spatial and temporal variance within and among the sex ratios of commercial landings, as determined via at-sea sex marking and genetic assays (2017-18).
7) Incorporation of sex-mark data collection into routine port sampling for commercial size and age data (2019 onward).

Brief summaries of the program's first four stages are found in the subsections that follow.

Development of at-sea marking methods

At-sea marking methods were developed and tested in 2014 by IPHC student intern Orion McCarthy, in a dual-phase study that began in the Alaskan ports of Homer and Dutch Harbor and was completed during the IPHC setline survey aboard the F/V *Kema Sue*. The objective of McCarthy's work was to develop a method for sex-marking halibut that would be easy for fishermen to accomplish while dressing their catch, would not damage their catch from a commercial perspective, and would allow our port samplers to easily and accurately distinguish between marks. Nine marks were initially tested portside, including cuts to various fins, the tail, and the gill plate. With feedback from the port samplers, fishers offloading the halibut, and local buyers, each potential mark was ranked according to its ease and practicality. From the original nine marks, the top three were then tested to determine which would be easiest for the port samplers to identify while also taking length data and collecting otoliths. The two "winning" marks were then used by the crew of the *F/V Kema Sue* to mark all retained catch from six days of survey fishing, during which the crew provided feedback on the ease of marking, and improvement in their marking accuracy through experience was evaluated by the intern through the trip. After retained fish were dressed and marked by the crew, and then sampled for biological information by the IPHC sea samplers, they were inspected by the intern for the presence/absence of the knife cuts and tagged with a unique fish identity number (ID). These unique fish ID numbers were matched with the sample data for each individual fish and used to keep a record of each fish's true sex, the sex marking, and where the fish was caught including station and skate number. During the offload, the IPHC port sampler in Dutch Harbor examined all the halibut in the catch and recorded the sex based on the mark as well as the individual fish ID. The sex ratio of the catch was estimated from the marks counted by the port sampler and compared with the sex ratio of the catch as marked by the crew as well as to the known true sex ratio for the trip.

The two marks that were chosen were as follows: for females, two cuts made in the dorsal (upper) fin; for males, a single cut through the white-side gill plate (Fig. 1a). The vessel crew marked ~85% of the catch correctly. Roughly two thirds of incorrectly-marked fish had either not been given a mark or were given a mark that couldn't be identified later; fewer than 5% of the fish were marked as the wrong sex. Ultimately, the proportion of female halibut in the offload as estimated from crew's sex-marks was ~3% greater than its known composition (i.e., 85% female versus a true proportion of ~82%), while the port sampler's estimate was ~5% low (i.e., 77% female). These results indicated that an at-sea sex-marking program would have considerable promise for providing sex-ratio data at the resolution required for assessment purposes, given that both accuracy and precision could be measured and monitored over time. This was especially true considering that the crew became more comfortable with the process and increasingly accurate as the trip progressed; suggesting that sex-marking should become easier and potentially more accurate than estimated as the project is scaled upwards and the fleet gains experience with it. For additional details regarding this project component, please see McCarthy (2015).

Pilot test of at-sea marking on commercial trips

During April and May of 2015, the IPHC's Homer port sampler, Jessica Marx, enlisted the cooperation of two vessels in the local fleet to conduct a voluntary field test of the marking method described above. Sex markings to accompany age and length data were obtained from 228 halibut representing five offloads. For each of these offloads, the crew marked all of their catch, but somewhat fewer total samples were obtained because IPHC port-sampling protocols may stipulate that not all fish from a given offload are to be sampled for age and length.

Feedback from the skippers and crew of the vessels regarding the ease of the process was positive, and a summary analysis of the sex ratio in their catches further highlighted the importance of collecting these data. Although the sample was relatively small and we are not yet able to verify the sex markings (for example, using the genetic technique described below), the data suggest that the vessels encountered a much higher proportion of female halibut across commonly-encountered ages than our setline survey data would have predicted based on similar sample sizes (Fig 2). This was most pronounced for halibut age 9-13, over all landed sizes. Whereas random samples of equivalent sample size and over the same age classes taken from the IPHC's Area 3A setline survey catches had been about 60-70% female, the commercial samples were 90+% female. Again, the sample is small and the marks unverified; however, it may be fair to suggest that these data are consistent with the notion that enough spatial and temporal variability may occur in the fishery to warrant closer monitoring using data that are not solely setline survey-based.

Voluntary at-sea marking by the Area 2B fleet

In advance of the 2016 commercial fishing period, IPHC staff met with representatives of the Pacific Halibut Management Association of British Columbia (PHMA) to discuss logistical considerations associated with a regulatory-area-wide voluntary sex marking program and to receive their input regarding the most efficient way to generate interest from the fleet. A laminated informational flyer (Figs. 1a & b) was produced to assist crew members in distinguishing between male and female Pacific halibut, and to describe the sex-marking procedure. The flyer was provided to PHMA who included it in their pre-season mailing to all Area 2B commercial license holders; i.e., 435 vessels. Subsequently, the IPHC's port samplers in Prince Rupert, Port Hardy, and Vancouver served to communicate and clarify the project's intent, answer any questions that fleet members might have, distribute reward hats to the crews of participating vessels to acknowledge their help with the project, and solicit their feedback as the season progressed.

Over the course of the season, 28 sex-marked landings were sampled representing just over 16% of the total number of landings and individual halibut sampled area-wide, and ~13% of those landings' total weight (Table 1). These samples represent just under 4% of 2B's 7.3 million pound (3,311 metric ton) catch limit: overall, a great level of participation for this phase of the project. Again, feedback from participants indicated that marking was not disruptive of normal fishing activity, nor did it have any adverse effects on marketability of these fish. At this time, these samples have not been completely aged (and are unverified via genetic testing) but they are scheduled for evaluation during 2017, the results of which will be reported in future summaries of this project.

Development of a genetic sex assay

We hope that fleet participation in at-sea sex marking will eventually be sufficient to generate the volume and quality of data required for assessment purposes. That said, because such marks will always contain some level of error we would need to periodically measure the accuracy and precision of the sex ratios that are estimated using the sex marks. To this end, it would be ideal to develop a genetic test for sex that can be used to routinely subsample the at-sea sex-marking samples. Although the development of genetic tests for sex in fishes can sometimes be difficult because their sex can be influenced by environmental factors such as temperature (Devlin and Nagahama 2002, Penman and Piferrer 2008), our earlier genetic research suggested that an accurate test for sex is likely possible for Pacific halibut. During the course of a population genetics study we discovered three genetic markers that are closely associated with sex in Pacific halibut (Galindo et al. 2011), and in 2016 began the process of developing a high-throughput assay that could be conducted at the IPHC's Seattle laboratory. Restriction-site associated DNA sequencing identified 56 genetic loci linked to sex, of which three have been identified to persist exclusively in females. Initial tests suggest that these markers will yield highly accurate sex identification, and work is currently underway to finalize the choice of markers and develop testing protocols. For additional details regarding this project component, please see Drinan et al. (2017). With full development of such an assay, validation of the at-sea sex marking could be conducted by obtaining a small piece of fin tissue, followed by an assay in the lab. The combination of at-sea sex marking (low cost) and genetic assay (larger cost) could then result in a cost effective means of estimating the sex distribution of the commercial catch.

Future timeline

In 2017 we will expand the voluntary at-sea sex-marking program to include all IPHC-sampled ports in all regulatory areas: i.e., St. Paul Island, Dutch Harbor, Kodiak, Homer, Seward, Juneau, Sitka, and Petersburg, Alaska; Prince Rupert, Port Hardy, and Vancouver, British Columbia; and Bellingham and Newport, Washington. In conjunction with increasing the scale of the sampling effort, we will also seek to enhance the project's incentive program. In addition to giving out hats for participation in the program, as was done in 2016, we intend to add products such as shirts or jackets to the suite of items available. These are likely to be awarded based on vessels' levels of participation and the relative accuracy of their sex-markings as compared to genetic validation of those samples. Our intent is to continue sampling at a voluntary coastwide level through 2018, providing a period during which the fleet can continue to provide feedback and the data that are generated can be analyzed to assess precision, accuracy, and identify trends in sex ratios that may occur in space and time. Our goal is to have routine collection of commercial sex data in place for the 2019 fishing season, composed of a combination of at-sea sex marking by the commercial fleet in conjunction with limited genetic validation. Whether at-sea sex marking of the catch would remain voluntary or require regulatory action will be evaluated at a later date, and will depend upon the quality and completeness of the data that a voluntary program is able to produce.

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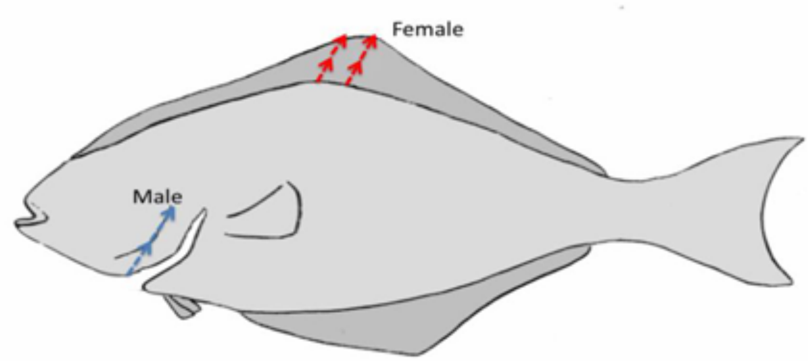
Table 1. Number of Pacific halibut fishery landings, weights of landed halibut, and number of biological samples taken (i.e., sagittal otoliths and accompanying tissue samples) by International Pacific Halibut Commission samplers at British Columbian (Regulatory Area 2B) ports during the 2016 commercial fishing season, both overall and composed of halibut that had been individually sex-marked aboard the fishing vessels at sea.

	Prince Rupert	Port Hardy	Vancouver	Total
Landings				
Sampled	47	76	7	130
Sex-marked	14	6	1	21
% sex-marked	29.8	7.9	14.3	16.2
Weight landed				
Sampled (pounds)	772,500	1,174,807	141,000	2,088,307
Sampled (metric tons)	350.40	532.88	63.96	947.24
Sex-marked (pounds)	178,000	81,500	15,000	274,500
Sex-marked (metric tons)	80.74	36.97	6.80	124.51
% sex-marked	23.0	6.9	10.6	13.1
Sex-marked: % of 2B Catch Limit	2.4	1.1	0.2	3.8
Samples taken				
Overall	1083	704	118	1,905
Sex-marked	272	45	13	330
% sex-marked	25.1	6.4	11.0	17.3

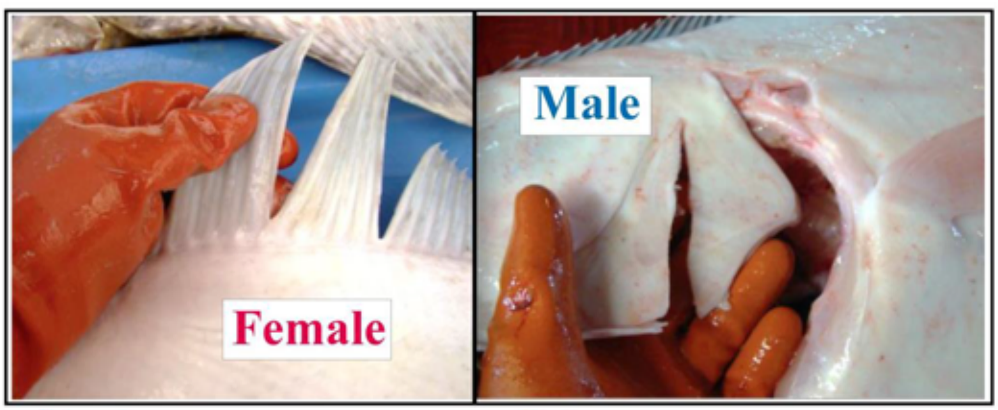
Then: Mark the fish as either female or male, using your gutting knife.



Locations on a halibut for marking it as either female or male

Female: Make two parellel cuts through the top (dorsal) fin (**see below, left**), being sure to make your cuts using an upward stroke, away from the animal, to avoid damaging the flesh. Two cuts must be made, so that the sex-marks cannot be confused with pre-existing injuries to the fin. Note that only the top (dorsal) fin can be marked; any marks found in the lower fin will be ignored when the fish is sampled in port.

Male: Make a single cut through the gill-plate (operculum) on the fish's white side (**see below, right**). Make the cut using an upward stroke, making the cut parallel to the rear edge of the operculum. The cut should extend about 3/4 of the way up the plate, so that the "flap" that you create will remain attached to the plate.



Female: Make two parallel cuts in the top (dorsal) fin.

Male: Make one cut through the white-side gill plate (operculum).

Please mark 100% of your catch!

Your effort is greatly appreciated!

Figure 1a. Page 2 of the laminated flyer distributed to the Area 2B fleet for the 2016 commercial fishing season, demonstrating how to mark halibut as either male or female while dressing them.

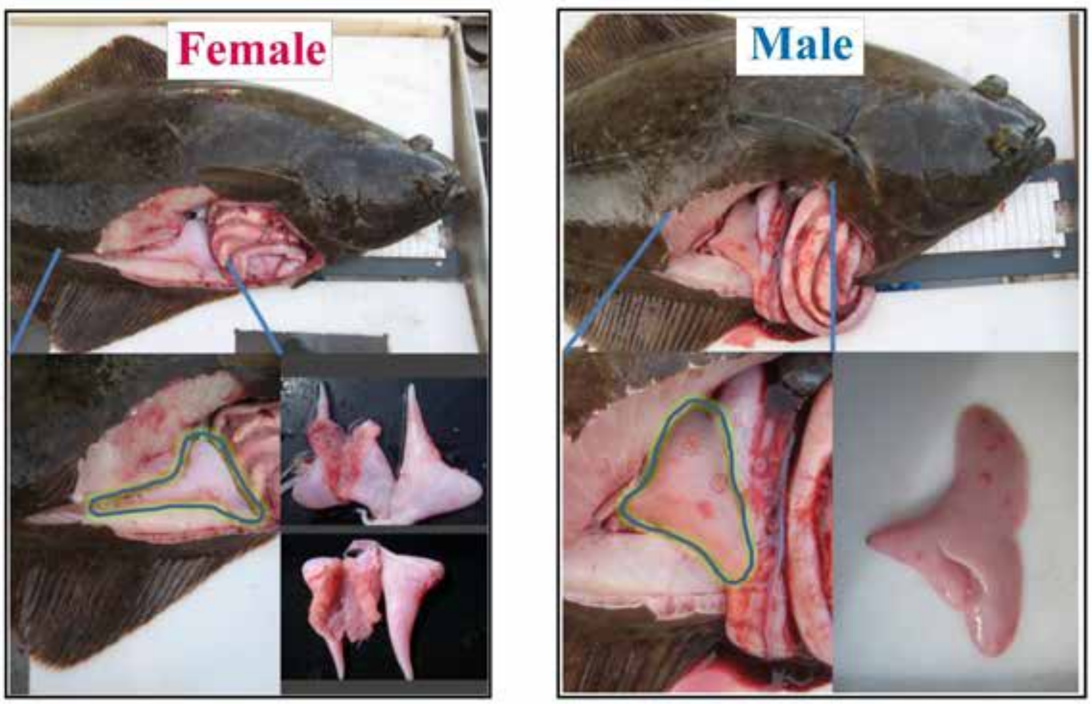
Sex-marking of halibut aboard commercial fishing trips

The IPHC requests your help during the 2016 fishing season, as we work to develop standard protocols for determining the sex of halibut that are landed by the commercial fishery. Accurate sex-ratio information is necessary for stock assessment - most notably, for accurately estimating and monitoring spawning stock biomass. You can help by marking the sex of the fish that you catch, while dressing them, using the identification-cuts that are described below.

First: Determine whether you have a female or a male halibut.

Female halibut have ovaries that are elongated (funnel-shaped) triangles (**see below, left**). These take up the rear portion of the gut cavity, farthest from the head, and extend back into to body. The ovaries are smooth and sac-like, with a blutly rounded front edge. Inside, the ovaries may contian develoiping eggs; the outer surface may have well-developed blood vessels. For fish of any given size, ovaries tend to be much larger than testes.

Male halibut have testes that are pale pink and relatively triangular (**see below, right**), with a sharly-tapered front edge, and lacking visible blood vessels on the outer surface. The testes are made up of overlapping lobes (a bit like a liver) that produce fine notches and crevices in the surface. They are also in the rear of the gut cavity, farthest from the head.



Female halibut: ovary location and shape. Ovaries have an elongated funnel-shape, and are a smooth sac with a rounded front edge.

Male halibut: testis location and shape. Testes are more triangular than ovaries, are composed of overlapping lobes, and have a sharper front edge.

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Figure 1b. Page 1 of the laminated flyer distributed to the Area 2B fleet for the 2016 commercial fishing season, describing the difference between female and male Pacific halibut.

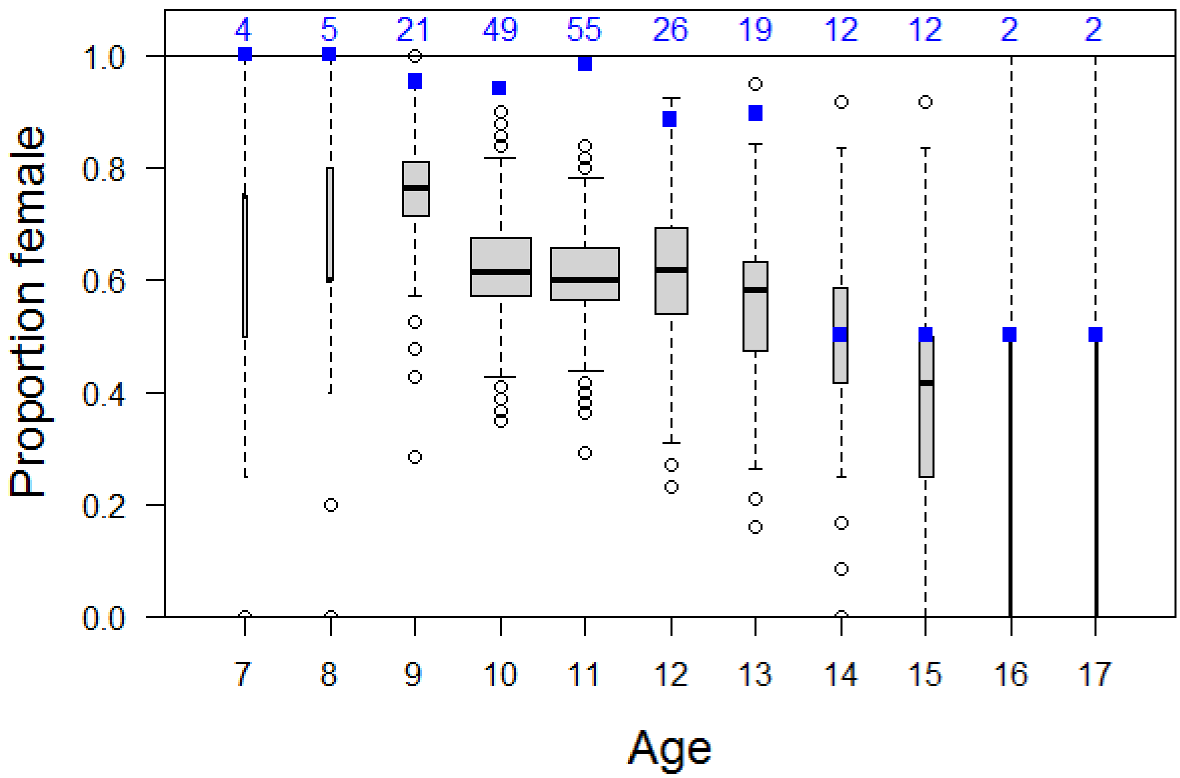

Figure 2. Based on voluntary at-sea sex marking of commercially-harvested Pacific halibut (n = 207) landed in Homer, Alaska in 2015, proportions of female halibut at-age within those landings (small blue squares) relative to what would have been expected from similar samples sizes based on results of the IPHC fishery-independent setline survey (box and whisker plots) during 2015 in Regulatory Area 3A. In the box and whisker plots, the horizontal lines indicate the median values; the gray boxes contains the central 50% of expected values around those medians; the dashed line the 95% interval; and the dots beyond the expected variation indicate unlikely-yet-possible "outlier" values. Sample sizes by age are denoted in the top margin. Note that for halibut <14 years of age, the sampled commercial trips were composed of considerably more females than would have been expected.