

## **Project Title: Testing a non-lethal method for determining the sex of California halibut, *Paralichthys californicus*, in non-spawning condition**

### **PROJECT GOALS:**

The primary goals of our project were to test a non-lethal method of determining the sex of California halibut (*Paralichthys californicus*) and to obtain samples in a way that avoids causing additional mortality to a significantly depleted resource. Secondary goals are to also build capacity for collaborative research with California Department of Fish and Wildlife (CDFW, formerly Fish and Game) in the Los Angeles Area, provide an educational opportunity for college and graduate students in the Los Angeles area, reinforce partnerships with the fishing community, and publish results as research notes in a peer reviewed journal.

### **METHODS:**

The accuracy of veterinary ultrasound was determined using gross morphological comparisons between live ultrasound sonography images and either visual inspection (in landed fish) or examination of genetic material (in aquacultured fish). The effects of reviewer experience and training were also assessed. To limit the impact of this study on the halibut population, 101 samples were obtained from the following sources: bycatch from commercial purse seine fleet in Santa Barbara (fish were caught 12/2012 and 1/2013, frozen and then thawed before examination on 3/14/2013, n = 34), recreational landings from the Santa Monica Bay as part of the Marina Del Rey Anglers annual halibut derby (6/8-9/2013, n = 13), recreational landings at the Marina Del Rey boat launch (7/28-29/2013, n=5), aquaculture stock from Hubbs-SeaWorld Research Institute (HSWRI; 4/16/2013, n = 30) and the Sea Lab in the Redondo Beach (4/23/2013, n = 19).

Of the 101 samples, 20 could not be used, leaving a total sample size of 81. The majority of the unusable samples were the frozen fish from the commercial fishery (n=13, due to archived image problems such as the wrong location, incorrect depth of field, or blurry)<sup>1</sup>. In addition, 2 fish from the Sea Lab did not have their sex confirmed because they were not mature enough and all 5 fish from the boat launch could not be used because ultrasound and/or visual confirmation was not obtained.

All fish were sampled outside the peak spawning period. However, halibut have an extended spawning period; therefore all samples were taken when moderate levels of spawning activity were possible (Moser & Watson, 1990).

Ultrasound sonography was performed using a refurbished Sonosite 180 veterinary ultrasound equipped with a 10 – 5 MHz, 38mm aperture linear transducer. Ultrasonic coupling gel was not used, as a good image was attained without it (see also (Loher & Stephens, 2011)). The transducer was oriented longitudinally over the fish's eyed-side gonad and centered on the primary mass of the gonad (immediately posterior to the visceral cavity) dorsoventrally (see Figure 1). The frequency (or scanning depth) was adjusted for the size of the fish. In California halibut, the

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<sup>1</sup> These fish were primarily used to identify the morphological features used to distinguish males from females in the ultrasound images. Stored images were later re-coded using three-of-four agreement.

gonads of both sexes are roughly triangular in shape and taper posteriorly between the caudal vertebral interhaemal spines and abdominal muscles. In mature fish, the posterior extent of the ovaries is further than the testes. Ovaries can be further identified by the anterior portion, which is rounded, and by their relatively uniform shape and homogeneous density. In contrast, the testes are more angular, the shape varies between individuals, and is of heterogeneous density (see Figure 2).

Live fish were anesthetized with tricaine mesylate (MS-222). Once sedated, they were removed from the water, weighed using a tarred flat scale, measured using a fish board (total length), and returned to a tub of fresh sea water to be scanned with the ultrasound. The sex determined by the ultrasound was recorded prior to verifying sex. At HSWRI, pit tags enabled verification via past spawning records. At the Sea Lab, fish were cannulated according to the procedures described by Ross (1984) and eggs or sperm were identified visually or under a microscope. At the fishing derby, fish were weighed using an International Game Fish Association certified scale in pounds and the weight converted to kilograms. Visual confirmation of sex was conducted by making an incision starting at the cloacae and cutting toward the tail along the ventral side of the fish approximately 5cm to expose the gonad. Otoliths were later extracted and given to CDFW for aging. Frozen fish were thawed in the lab prior to examination, weighed and measured in the same manner as the live fish. Images were stored and used later for re-coding. After scanning with the ultrasound, sex was verified using the same method as for the fish caught in the halibut derby.

To determine the effect of reviewer experience and training, four reviewers of varying levels of experience re-coded saved images from a subset of the fish. Each reviewer was given a guidance document and a series of images to be re-coded. The image order was randomized for each reviewer. Reviewers had the option of coding the fish as male, female, or unclear based on the images provided. The accuracy of each reviewer was compared. In addition, the accuracy of the sexing based on three-of-four and four-of-four agreement was also compared. Disagreement between reviewers was also coded as unclear.

## RESULTS:

Of the 81 fish used in the study, 34 were legal size, 47 were sublegal, and they ranged in size from 401 mm to 1090 mm. Female halibut begin maturing at 360 mm, half are mature at 470 mm, and all are mature by 590 mm. Male halibut begin maturing at 190 mm, half are mature at 230 mm, and all are mature by 320 mm (Love & Brooks, 1990; Maunder, Reilly, Tanaka, Schmidt, & Penttila, 2011). There were a total of 44 females in the sample, ranging in size from 404 – 1090 mm; 17 were definitely mature, 17 were likely mature, 10 were likely immature, and none were definitely immature. There were a total of 37 males in the sample, ranging in size from 401 – 683 mm; all 37 were definitely mature (Table 1).

The ultrasound method only resulted in one misidentification of sex (98.8% accurate). This fish was a sublegal, but likely mature female (540mm) from HSWRI. Interestingly, this fish was correctly identified when using three-of-four agreement.

It was found to be more difficult to accurately diagnose sex when looking at saved rather than live images. The same reviewer has an overall accuracy of 98.8% (n=81) when scoring live

images vs an overall accuracy of 96.8% (n=62) when scoring from saved images. This is due to the added information gained from manipulating the transducer over the gonad.

When comparing reviewers, accuracy increased with experience<sup>2</sup> from 90.2% for the reviewer who had only looked at the training slide show to 96.8% for the reviewer who had been conducting the live ultrasounds. However, the number of fish the reviewer coded as unclear ranged between 0 and 8.6% and was not related to experience. Both three-of-four agreement and four-of-four agreement resulted in 100% accuracy (57/57 and 47/47 correct, respectively). However, a higher proportion were coded as unclear using four-of-four agreement (16/63 or 25.4%) than when using three-of-four agreement (6/63 or 9.5%). Therefore, three-of-four agreement is sufficient to obtain accurate results while maximizing the number of useable samples, when using saved images (Table 2).

#### COMMUNITY INVOLVEMENT:

This project engaged a research facility (HSWRI), educational institutions (LMU, Sea Lab), a recreational fishing group (MDRA), and a management agency (CDFW). The project facilitated a new relationship between SMBRF and HSWRI that may allow for future collaboration. It also added to the range of projects we have engaged in with the SEA Lab in Redondo Beach.

Capacity for collaborative research with CDFW and MDRA has been expanded. Immediate proof of this is that SMBRF and MDRA will be partnering on a new project related to halibut (developing methods for citizen scientists to collect essential fishery information) in collaboration with CDFW. This \$25,000 project was funded by the Collaborative Fisheries West and will begin in October of 2013.

#### OUTREACH ACTIVITIES:

Thirty-one College Students participated in data collection and learned about essential fishery information, fishery management, and this project.

#### CONCLUSION:

Ultrasound is a highly accurate method for determining sex in halibut > 400mm. Live ultrasound is easier to read and therefore more accurate than relying on saved images, reviewer accuracy improves with experience, and three-of-four agreement when reviewing saved images is sufficient to obtain accurate results while maximizing sample size. This technique will enable the California Department of Fish and Wildlife to expand their sex-specific data set on California halibut and other researchers to conduct sex-specific studies using live animals, such as tag-recapture studies, that will improve the reliability of the next stock assessment.

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<sup>2</sup> Hours of experience are not reported because they were not tracked.

FIGURES AND TABLES:

Figure 1. Position of ultrasound transducer along longitudinal axis of the halibut's eyed-side gonad and centered on the primary mass of the gonad immediately posterior to the visceral cavity.



Figure 2. A) Visual image of female gonad. B) Visual image of male gonad. C) Ultrasound image of female gonad. D) Ultrasound image of male gonad.



A)



B)



C)



D)

Table 1. Maturity of the sampled halibut by sex.

	<b>Immature</b> Male < 190mm Female < 360 mm	<b>Likely Immature</b> Male 190-229mm Female 360-469mm	<b>Likely Mature</b> Male 230-319mm Female 470-589mm	<b>Mature</b> Male > 320mm Female > 590mm	<b>TOTAL</b>
MALES	0	0	0	37	37
FEMALES	0	10	17	17	44
<b>TOTAL</b>	<b>0</b>	<b>10</b>	<b>17</b>	<b>54</b>	<b>81</b>

Table 2. Reviewer accuracy with experience and method accuracy using three-of-four and four-of-four agreement.

Experience	High	Medium	Low	None	3/4 Agreement	4/4 Agreement
Reviewer	1	2	3	4		
Sample Size	63	63	63	63	63	63
# Skipped	1	5	0	2	6	16
# Answered	62	58	63	61	57	47
# Correct	60	56	58	55	57	47
% Correct	96.8%	96.6%	92.1%	90.2%	100%	100%
% Unanswered	1.6%	7.9%	0.0%	3.2%	9.5%	25.4%

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