

Abstract—Determining the sex of thornyheads (*Sebastolobus alascanus* and *S. altivelis*) can be difficult under field conditions. We assessed our ability to correctly assign sex in the field by comparing results from field observations to results obtained in the laboratory through both macroscopic and microscopic examination of gonads. Sex of longspine thornyheads was more difficult to determine than that of shortspine thornyheads and correct determination of sex was significantly related to size. By restricting the minimum size of thornyheads to 18 cm for macroscopic determination of sex we reduced the number of fish with misidentified sex by approximately 65%.

Accuracy of sex determination for northeastern Pacific Ocean thornyheads (*Sebastolobus altivelis* and *S. alascanus*)

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Accurate sex-specific data are essential for fitting age-structured population dynamic models and estimating spawning biomass (Methot, 2000). Assessing sex ratio is of added importance if sex-based selectivity occurs within a fishery; because separate management measures may be required for male and female fish (Cochrane, 2009).

Thornyheads are a common continental slope species and support a large commercial fishery (Gunderson, 1997). Longspine thornyheads (*Sebastolobus altivelis*) are found from the Gulf of Alaska to southern Baja California, whereas shortspine thornyheads (*Sebastolobus alascanus*) are distributed from the Bering Sea to northern Baja (Orr et al., 2000). Longspine thornyheads generally inhabit depths greater than 400 m, have a distribution range to about 1400 m depth (Jacobson and Vetter, 1996), and a peak in abundance and spawning biomass at about 1000 m depth (Wakefield, 1990; Jacobson and Vetter, 1996). Shortspine thornyheads are found from 20 m to over

1500 m in depth, are most abundant in the range of 180 to 450 m, and the majority of the spawning biomass occurs between 600 and 1400 m, where longspine thornyheads are most abundant (Jacobson and Vetter, 1996). The maximum size of shortspine thornyheads (>70 cm) is larger than that of longspine thornyheads (~38 cm). Shortspine thornyheads migrate to deeper water as their body size increases, whereas longspine thornyheads do not migrate to deeper water with increasing size.

Identifying the sex of mature longspine thornyheads and shortspine thornyheads by gross visual examination is difficult when gonads regress to a resting state (Pearson and Gunderson, 2003) because male and female gonads are small, not fully developed, and are morphologically similar. Determining the sex of individual thornyheads collected during the annual Northwest Fisheries Science Center (NWFS) West Coast Groundfish Bottom Trawl Survey is difficult because the survey occurs

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from May to October when thornyheads are not reproductively active and gonads are in a resting state (Moser, 1974; Wakefield, 1990).

The addition of sex identification for both thornyhead species to survey sampling protocols will improve the information available for management of the resource. To address concerns about the ability of field personnel to correctly determine sex of thornyheads while at sea, we examined the sex of longspine and shortspine thornyheads in the laboratory using macroscopic examination of gonads (as a correlate for field work) in contrast to microscopic techniques (for confirmation of results). An additional goal was to determine a minimum size below which the error rate for classification of sex of thornyheads in the field was judged to be too high by investigating the relationship between sex misidentification and length, geographic area, and month captured. Because assessment scientists are interested in the actual proportion of males to females, we also evaluated absolute percent error after accounting for the portion of the error that was cancelled out by balancing the number of misidentified males reported as females against the number of misidentified females reported as males.

Materials and methods

The 2003 NWFSC West Coast Groundfish Bottom Trawl Survey was conducted between 24 June and 23 October, from the area off Cape Flattery, Washington (48°10'N lat.) to the U.S.-Mexico border (32°30'N lat.) at water depths of 55–1280 m. The survey area was covered twice by chartered commercial fishing vessels (20 to 28 m length). The first sampling period was from 24 June to 13 August and the second from 31 August to 23 October. A stratified random sampling design was used and the survey area was subdivided into adjacent cells of equal area (1.5 nmi long. by 2.0 nmi lat., Albers equal area projection). A total of 620 primary sites were randomly selected from cells stratified by geographic location and depth. The geographic allocation was based on assigning 15–25% of the cells to each of five International North Pacific Fisheries Commission (INPFC) statistical areas: U.S.-Vancouver (47°30'N to U.S.-Canada border), Columbia (43°00' to 47°30'N), Eureka (40°30' to 43°00'N), Monterey (36°00' to 40°30'N), and Conception (U.S.-Mexico border to 36°00'N). The survey area was further stratified into depth zones with 45% of the cells allocated to the shallow depth zone (55–183 m), 30% to mid-depth (184–549 m) and 25% to the deep stratum (550–1280 m). Each of four chartered fishing vessels was assigned 155 stations to sample.

The bottom trawl survey is a standardized fishery independent survey and all fishing operations are conducted in strict compliance to national protocols (Stauffer, 2004). Vessels were equipped with standard Aberdeen-style nets with small mesh (1.5-inch stretched measure) liner in the codend. All thornyheads randomly selected for biological sampling were assigned a unique identi-

fication number, individually weighed (kg), measured (fork length, cm), and frozen while at sea. All frozen specimens were brought back to the laboratory where fish were thawed, dissected, and examined macroscopically to identify sex. For macroscopic examination of gonads, an incision was made with a scalpel on the ventral surface of each thornyhead from the vent to the base of the pectoral fin. The lateral side of the fish was opened to expose the gonads, and a visual identification of sex was based on the physical structure of the gonadal tissue as described by Lagler et al. (1962). Sex was recorded as male, female, or unknown. For microscopic identification of sex, a section of gonad tissue from each fish was placed on a glass microscope slide, stained with acetocarmine solution and compressed with a cover slip. The stain acted on the gonad tissue by readily staining oocytes dark pink (Guerrero, 1974). The slides were viewed under a 10× power microscope (Leica DM LS2, Bannockburn, IL), and females were distinguished from males by the presence of dark pink stained oocytes.

Accuracy of sex determination was examined in relation to length by species, geographic region, and month of capture (June–October). To determine a size threshold below which sex determination should not be attempted in the field, we examined both the total and absolute percentage of incorrectly sexed thornyheads in relation to length. To avoid biasing results, we did not consider our ability to correctly identify female thornyheads at smaller sizes, as opposed to our ability to correctly identify males at smaller sizes. Absolute error was calculated as the absolute value of misidentified males minus misidentified females divided by the total number examined at each 1-cm size interval, and this value was then expressed as a percentage. Size data were transformed (natural logarithm) to reduce heterogeneity of variance before statistical analysis. Data were statistically compared by analysis of variance (ANOVA) by using SAS for Windows (SAS Institute, Inc., Cary, NC). Significant ANOVAs were followed by a nonparametric comparison of means test (Tukey's test). Fish in which the gonad could not be found, stained, or microscopically identified were not included in the analyses.

Results

A total of 574 successful tows were completed. Figure 1 shows the distribution and relative abundance (kg/ha) of thornyheads from the 2003 survey. Both species were concentrated in the mid- and deep depth strata (183–1280 m) and exhibited higher relative abundance north of Pt. Conception, CA (34°30'N lat.). Longspine thornyheads were collected in 214 tows at depths of 328–1280 m (mean depth 802 m) and shortspine thornyheads were collected in 311 tows at depths of 88–1280 m (mean depth 605 m). A total of 2325 thornyheads were collected for later processing in the laboratory. Sex was determined for 852 longspine thornyheads and 1148 shortspine thornyheads. Sex was indeterminable for 189 longspine and 136 shortspine thornyheads (average

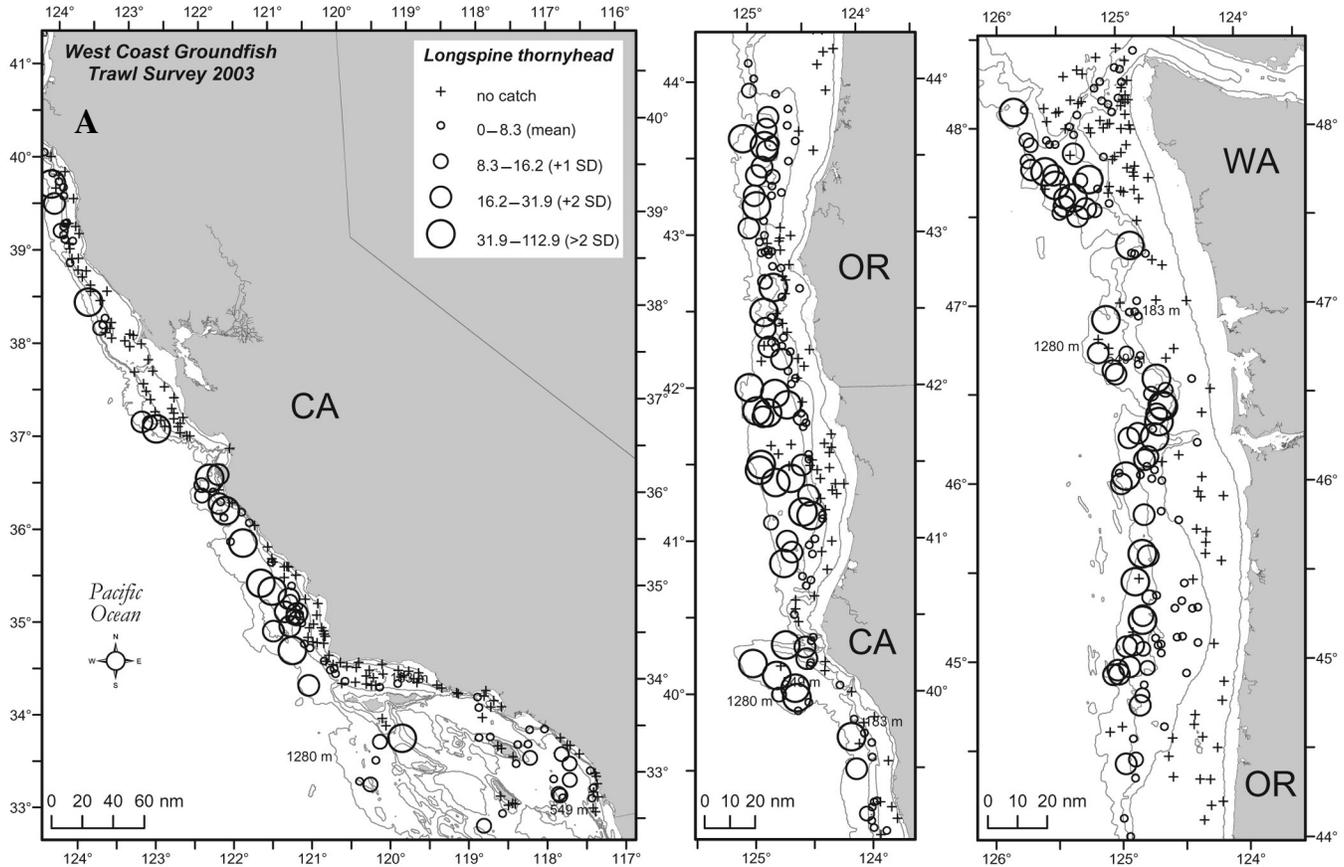


Figure 1

Distribution and relative abundance (kg/ha) of (A) longspine thornyhead (*Sebastolobus altivelis*) and (B) shortspine thornyhead (*Sebastolobus alascanus*) determined from the 2003 Northwest Fisheries Science Center west coast groundfish trawl survey. SD=standard deviation.

length 14.3 cm). Longspine thornyhead sex was misidentified by visual examination in 23.1% of males and 22.4% of females, and for shortspine thornyheads, in 9.4% of males and 9.3% of females.

Average lengths of longspine and shortspine thornyheads (females, males, and total) for which sex was misidentified were significantly lower than the lengths for fish whose sex was correctly assigned (Table 1). For shortspine thornyheads, the average length of sex-misidentified females was significantly smaller than that of males (ANOVA: $df=6$, $F=5.5$, $P=0.02$). Similar tendencies were seen for longspine thornyhead lengths but the results were not significant (Table 1).

Determining sex for longspine thornyheads greater than 22 cm would eliminate approximately 80% of the overall error rate, but would also eliminate 50% of the fish whose sex was correctly determined. By proposing 18 cm as the minimum size for examining longspine thornyheads in the field we eliminated approximately 65% of the incorrectly sexed fish, while retaining >70% of those correctly sexed (Fig. 2A). On average, the sex of 50.5% of longspine thornyheads ranging in size from 11 to 17 cm was incorrectly determined. This average dropped to approximately 10% for longspine thorny-

heads at lengths from 18 to 34 cm. A similar result was seen for shortspine thornyheads (Fig. 2A). The average percentage of shortspine thornyheads with misidentified sex was 53.7% at lengths from 11 to 17 cm. This value decreased to 5.9% for larger fish (18–71 cm) (Fig. 2A).

With a single exception, more males were misidentified as females in every size category for both species, and the absolute percentage of sex-misidentified fish decreased at fork lengths greater than 17 cm (Fig. 2B). For longspine thornyheads the average decreased from 15.8% for fish 11–17 cm to 2.2% for fish 18–34 cm length and the average percentage for shortspine thornyheads dropped from 24.5% to 3.0% in the larger size category (Fig. 2B).

Sex misidentification in longspine thornyheads did not vary significantly by month from June through October (ANOVA: $df=7$, $F=1.74$, $P=0.34$; Fig. 3A). However, sex misidentification for shortspine thornyheads was significantly higher in August, with an increasing trend from June through August followed by a decline (ANOVA: $df=7$, $F=15.5$, $P=0.02$; Fig. 3A).

The accuracy of sex determination varied by geographic area for both species (Fig. 3B). The sex of longspine thornyheads was more frequently misidentified

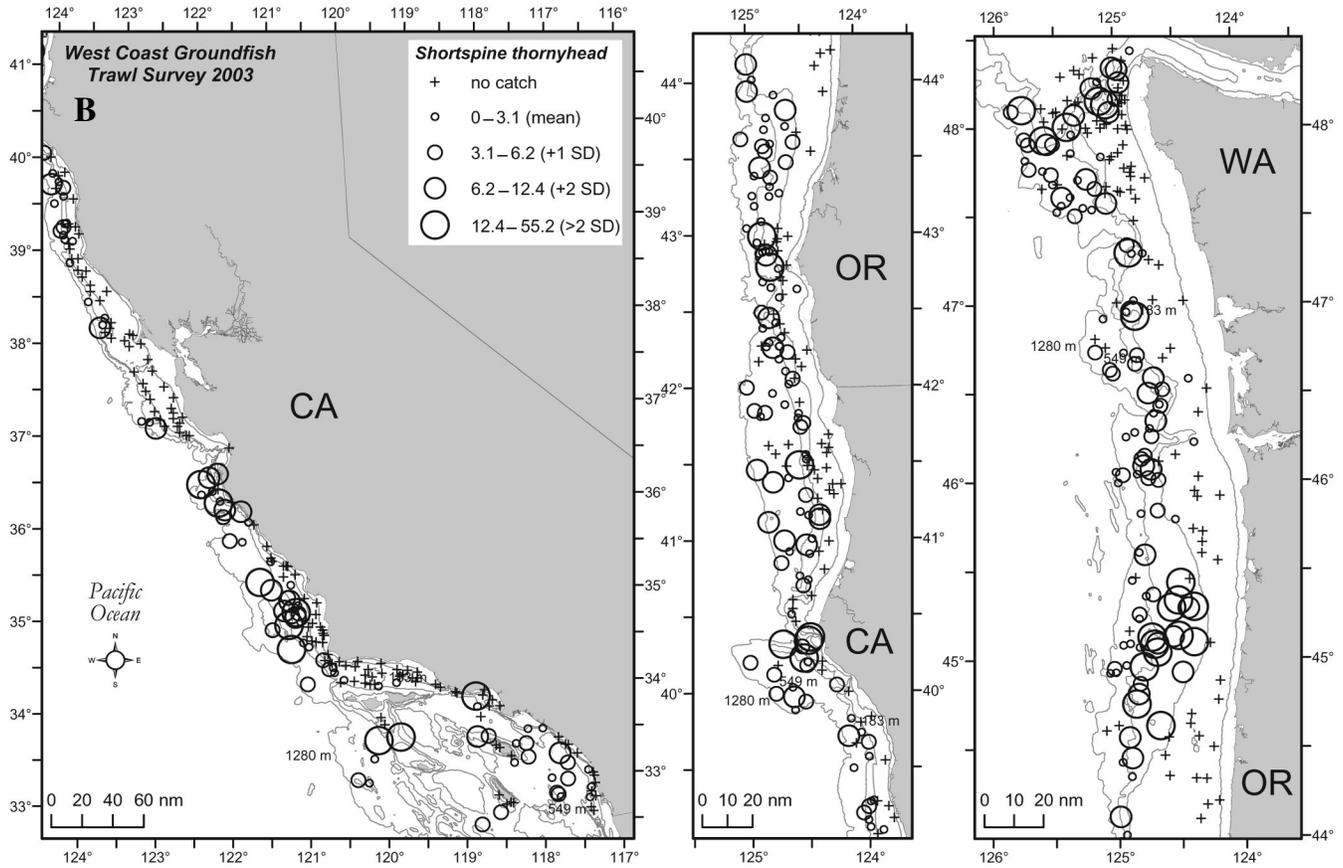


Figure 1 (continued)

Table 1

Number (*n*), mean fork length (cm, ±standard error [SE]), and analyses of variance (ANOVAs) for sizes for female, male, and total longspine (*Sebastolobus altivelis*) and for female, male, and total shortspine thornyheads (*S. alascanus*) captured during the 2003 Northwest Fisheries Science Center west coast groundfish trawl survey, correctly and incorrectly assigned sex based on visual examination.

Species	Correct		Incorrect		ANOVAs		
	<i>n</i>	Mean length (±SE)	<i>n</i>	Mean length (±SE)	df	<i>F</i>	<i>P</i>
Longspine thornyhead							
female	396	21.6 (0.21)	114	18.5 (0.37)	509	48.9	0.0001
male	259	23.4 (0.20)	83	19.0 (0.47)	341	96.6	0.0001
total	655	22.3 (0.15)	197	18.7 (0.29)	851	52.7	0.0001
Shortspine thornyhead							
female	560	35.5 (0.52)	58	23.6 (1.16)	617	50.8	0.0001
male	481	34.9 (0.45)	49	28.1 (1.52)	529	21.5	0.0001
total	1041	35.2 (0.35)	107	25.7 (0.96)	1147	36.2	0.0001

above 43°N latitude, and the U.S.-Vancouver and Columbia areas had a significantly higher average percentage of misidentification than the Eureka, Monterey, and Conception areas (ANOVA: *df*=4, *F*=44.1, *P*=0.007). The sex of shortspine thornyheads became more diffi-

cult to correctly identify below 40°N latitude, and both the Monterey and Conception areas had a significantly higher average percentage of misidentification compared to the Eureka, Columbia, and U.S.-Vancouver areas (ANOVA: *df*=4, *F*=13.9, *P*=0.03). There were no

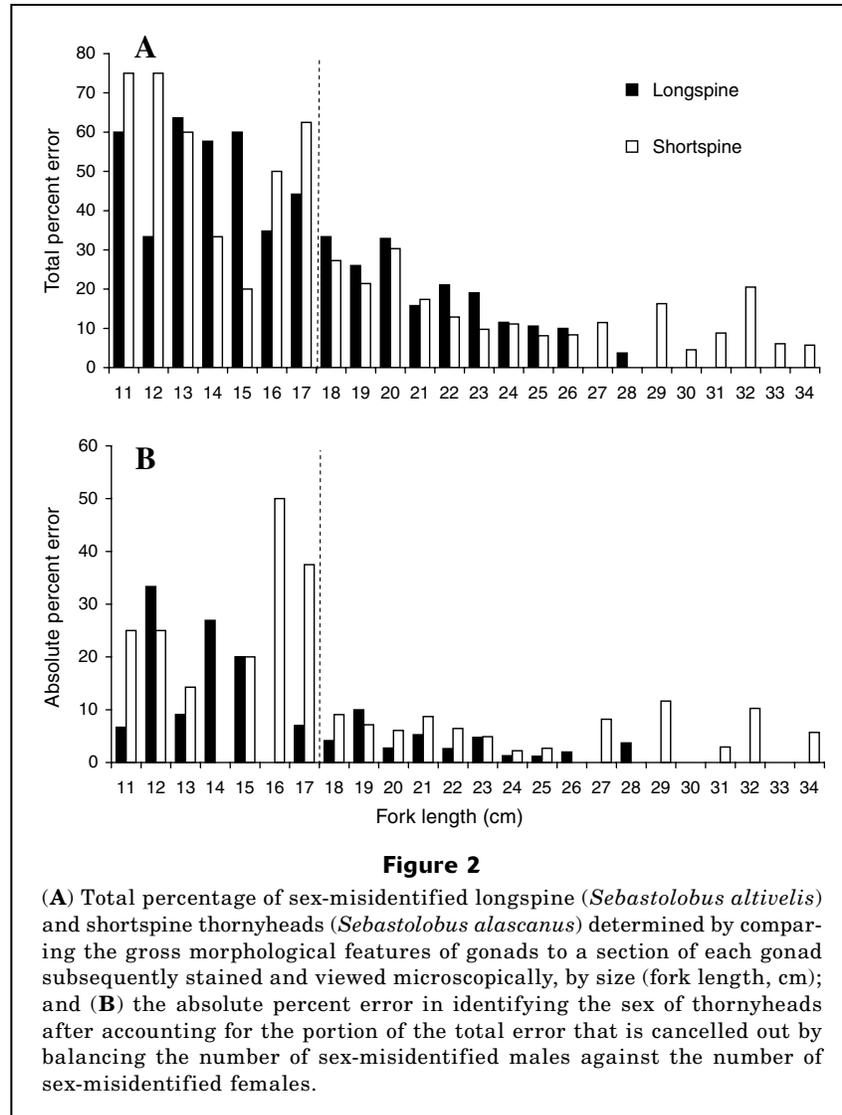


Figure 2
(A) Total percentage of sex-misidentified longspine (*Sebastolobus altivelis*) and shortspine thornyheads (*Sebastolobus alascanus*) determined by comparing the gross morphological features of gonads to a section of each gonad subsequently stained and viewed microscopically, by size (fork length, cm); and **(B)** the absolute percent error in identifying the sex of thornyheads after accounting for the portion of the total error that is cancelled out by balancing the number of sex-misidentified males against the number of sex-misidentified females.

significant differences in mean fork length for longspine thornyheads between the different areas (ANOVA: $df=858$, $F=0.3$, $P=0.9$), but for shortspine thornyheads, size was significantly larger in the Monterey and U.S.-Vancouver areas (ANOVA: $df=1140$, $F=4.7$, $P=0.0009$), and large fish in the Monterey area had a higher rate of individuals for which sex was incorrectly determined than similar size shortspine thornyheads in the U.S.-Vancouver area.

Discussion

This study provides guidance for a minimum size limit below which sex of thornyheads should not be determined at-sea because of high error rates. High quality biological information is important for management and modeling of thornyhead populations along the U.S. west coast (Fay, 2005). Fishery scientists need estimates of

sex ratio for fish populations because shifts in these values can indicate overfishing on one sex or the other due to selective gear, differential growth rates, segregation by sex or any combination of these (Cochrane, 2009).

In previous studies of the reproductive biology of thornyheads, the longspine thornyhead spawning was determined to begin in January, peak in February and March, and continue at least through April (Wakefield, 1990; Pearson and Gunderson, 2003; Cooper et al., 2005). Shortspine thornyheads spawn between December and May along the U.S. west coast. The onset of sexual maturity occurs at 17–19 cm total length (10% mature females) in both species and 90% are mature at 25–27 cm (Pearson and Gunderson, 2003). Sex of smaller thornyheads is difficult to determine, particularly during the summer, because of the small size of the gonads—size being a function of the annual spawning cycle. Pearson and Gunderson (2003) noted that of 36 longspine thornyheads designated as immature

females in the field on the basis of gross morphological features, nine were actually males.

Correct visual identification of sex for both shortspine and longspine thornyheads increased in fish longer than 17 cm. Overall accuracy is greater for shortspine than for longspine thornyheads, and greater for females than for males, and this accuracy is related to size in both instances. For both species, 18 cm was selected as the lower limit for determining the sex of thornyheads in the field because the majority of sex-misidentified fish fell below this value. In 2003, 66% of the longspine thornyheads and 90% of the shortspine thornyheads measured in the field throughout the survey period were greater than 17 cm. The selected size falls within the range of lengths noted for the onset of sexual maturity in both species.

Because the survey is conducted after the completion of the spawning season for longspine thornyheads (January–April), the samples are collected exclusively during the reproductive resting stage. Sex misidentification was relatively constant for longspine thornyheads throughout the sample period and there were no significant differences among months. Sex misidentification was greater for longspine than for shortspine thornyheads for each time period. The lower rate of sex misidentification for shortspine thornyheads may be related to their longer spawning season (December–May). Differences in the reproductive cycles of the two species resulted in the cessation of spawning coinciding with the start of the survey sampling for shortspine thornyheads and may partially explain the observed overall lower rate of sex misidentification for this species. The middle of the reproductive resting-stage period correlated with high levels of sex misidentification for both species, although only for shortspine thornyheads was the difference significant (in August).

The differences in sex misidentification among geographic areas are more difficult to explain. Sex of longspine thornyhead was more frequently misidentified in the U.S.-Vancouver and Columbia areas. Samples in these areas were collected primarily in June and September, the periods with the highest rates of sex misidentification. The lack of any significant differences in mean length for longspine thornyheads between INPFC areas indicates that the higher rates of misidentification of sex farther north were not a function of size, but were related to the timing of the annual spawning cycle at differing latitudes.

Shortspine thornyhead samples collected in the Eureka, Columbia, and U.S.-Vancouver areas (i.e., those with significantly lower rates of sex misidentification) were primarily taken in June, July, and September when the rate of sex misidentification for shortspine thornyheads was lowest. Additionally, there were significant differences in the lengths of shortspine thornyheads among areas, indicating that the lower rates of sex misidentification in the U.S.-Vancouver area may also be partially related to size (although similar size differences were not observed in the Eureka and Columbia areas). Because differences in geographic area were related to size

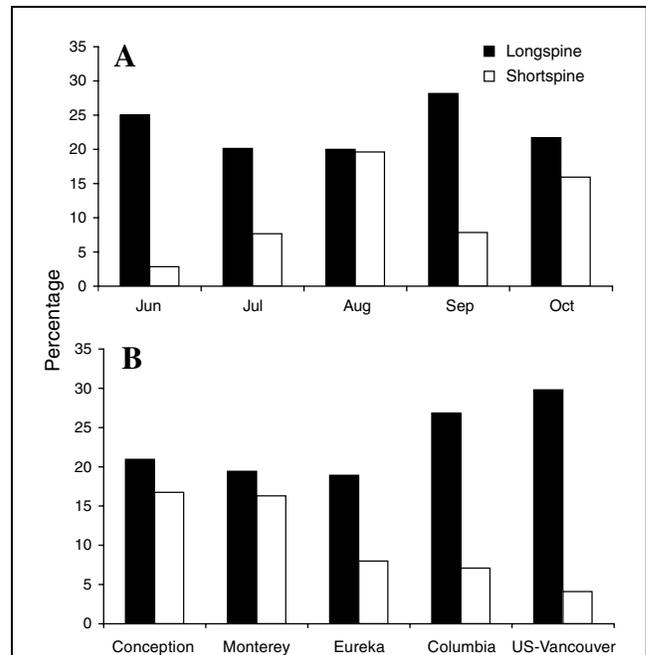


Figure 3

Percentage of sex-misidentified longspine (*Sebastolobus altivelis*) and shortspine (*Sebastolobus alascanus*) thornyheads determined by comparing the gross morphological features of gonads to a section of each gonad subsequently stained and viewed microscopically (A) by month, and (B) by geographic area as defined by the International North Pacific Fisheries Commission regions: Conception (U.S.-Mexico border to 36°00'N lat.), Monterey (36°00' to 40°30'N lat.), Eureka (40°30' to 43°00'N lat.), Columbia (43°00' to 47°30'N lat.), and U.S.-Vancouver (47°30'N lat. to U.S.-Canada border).

for at least one thornyhead species and the differences in seasonal determination of sex were variable, we recommend that sex determination of thornyheads <18 cm not be attempted in the field. This is likely a conservative estimate because identifying sex in fresh specimens at sea is somewhat more reliable than examining frozen and thawed specimens in the laboratory. The approach described here establishes a protocol for determining a minimum size for at-sea sex identification of thornyheads, but may be applicable for use with any species where ambiguity may exist in correctly identifying the sex of fish at smaller sizes, within different regions, or across spawning or other seasonal cycles.

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